# *Transplantation of Tissues*

SKIN, CORNEA, FAT, NERVES, TEETH, BLOOD VESSELS, ENDOCRINE GLANDS, ORGANS, PERITONEUM, CANCER CELLS

Edited by

Lyndon A. Peer, M D

Clinical and Research Director Rehabilitation Center for Plastic and Reconstructive Surgery, Saint Barnabas Medical Center Newark, N J

with Twelve Contributors

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Volume II

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*Cartilage Bone Fascia, Tendon, and Muscle*

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Vol. II. Transplantation of Tissues
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## DEDICATION

To the memory of Dr Milton Adams, whose untimely death is a great personal loss. He contributed in a large measure to plastic surgery and enriched life for all who enjoyed his friendship.

# *Contributors*

STERLING BUNNELL, M.D
Late Consultant to the Surgeon General of United States Army to the United States Navy and to the Alaska Department of Health

RAMÓN CASTROVIEJO M D
Clinical Professor of Ophthalmology New York University Post-Graduate Medical School Chief of the Department of Ophthalmology St. Vincent s Hospital and Director of the Corneal Clinic of New York City Eye and Ear Infirmary New York N Y

MIKLOS CSEREPFALVI, M.D., D.D.S.
Beneficiary of Rockefeller Foundation s Program for Aid to Hungarian Refugees Seeking to Establish Themselves in the United States Saint Barnabas Medical Center Newark N J

RALPH A DETERLING JR. M.D
Associate Attending Surgeon, Presbyterian Hospital, New York Associate Clinical Professor of Surgery College of Physicians and Surgeons, Director of Surgical Research Laboratories Columbia University New York, N Y

E J EICHWALD M.D
Director of the Laboratory of Experimental Medicine Montana Deaconess Hospital Great Falls, Mont.

HAROLD S FLEMING D.M.D M.Sc D.Sc.
Research Associate Department of Pathology Yale University School of Medicine New Haven, Conn

DAVID M HUME, M.D
Professor of Surgery and Chairman of the Department, Medical College of Virginia Richmond, Va

ABDOL H ISLAMI M D
Assistant Director of the Post-Graduate Department and Director of the Division of Experimental Surgery Seton Hall College of Medicine and Dentistry Consultant in Experimental Surgery Saint Barnabas Medical Center Newark N J

P L. KROHN
Nuffield Senior Gerontological Research Fellow and Honorary Professor University Medical School, University of Birmingham Birmingham England.

P B MEDAWAR, D.Sc
Jodrell Professor of Zoology University College, University of London.

MARY S PARSHLEY Ph.D
Research Associate in Surgery College of Physicians and Surgeons, Columbia University New York N Y

LYNDON A PEER, M.D
Clinical and Research Director Rehabilitation Center for Plastic and Reconstructive Surgery Saint Barnabas Medical Center Newark N J

BLAIR OAKLEY ROGERS M.D
Instructor in Plastic Surgery New York University College of Medicine New York University Bellevue Medical Center Consultant Tissue Culture Department Saint Barnabas Medical Center Newark, N J

# *Preface*

The wide variety of transplants discussed in this second volume i.e., organs, endocrine glands, cornea, cancer cells, etc. has necessitated a group presentation since no single individual has more than a general knowledge concerning the behavior of all these tissues and their clinical application. My colleagues, however have conformed to the same general pattern of presentation as in Volume I. Thus experimental work on animal tissue is separated from experiments with humans, since the two are not always the same.

The clinical application of research findings is again emphasized so that practitioners may take from the text suggestions of practical value in medical or surgical care. An example of such practical application is the clinical use of diced cartilage grafts, which is described in Volume I. These grafts when investigated experimentally were found to be extremely valuable in the repair of persistently recurrent hernias in cases where tantalum mesh fragmented, and dermal and fascial grafts proved to be inadequate in preventing recurrence. Although diced cartilage is better than dermal grafts for severe types of recurrent hernia, it is not as suitable as buried dermis for the repair of other conditions, which are described under clinical uses of skin.

Each contributor to this volume describes how tissue, organ or gland transplants may be grafted and the success, limitation or failure of the procedures.

Unfortunately many physicians tend to regard the study of tissue structure and cell behavior in free grafts as a somewhat impractical subject, detached from clinical medicine and surgery. In fact the exact opposite is true as evidenced by the many valuable contributions arising from studies of cell behavior which are in general use today.

One cannot overemphasize the importance of understanding the behavior of autografts, as described in Chapter 1 "Cells and Tissues." This is not only an important standard in evaluating the fate of homografts but also provides a basis for successful clinical use of the patient's own tissue. Statements that "the behavior of autografts is well known" are extremely optimistic, and further study is required to clear up controversial aspects of the subject. This may result in more satisfactory clinical management of for example free nerve, peritoneum, fat, muscle and preformed autogenous blood vessel grafts.

The surgical need of shifting or transplanting tissue from sound parts of the body to a defective part is fundamental, but in some patients normal tissues cannot be spared. Grafts have therefore been taken from other living individuals, from recently deceased healthy young adults killed in accidents or from still-born infants (embryonal tissue).

This rapidly expanding field of homografting, which appeared fanciful in the past, has a growth potential which should not be ignored. It is generally believed that the behavior of skin homografts indicates the behavior of other soft tissue homogenous transplants. Emphasis has therefore been placed on skin, the behavior of which is easy to follow because it is on the surface of the body. Recent work demonstrating the long survival of skin grafts exchanged between mother and child * and the possibility of rendering human embryos or newborn infants tolerant to a parent's skin by an injection of living cells from the parent offer new avenues of approach for successful homografting. Children who tolerate their mother's skin may also tolerate other tissues such as endocrine glands, cartilage and kidney. It is now established that homografts exchanged between identical twins or transplanted to individuals with agammaglobulinemia behave like autografts. These and other experimental findings indicate that successful homografting with a wider variety of tissues may soon be possible. Heterografting is still in the field of experimental surgery but homografting of blood, cornea, cartilage, bone and blood vessels is an accepted procedure.

Grants from The John A. Hartford Foundation and The Victoria Foundation were of material aid in furthering these research studies.

Transplantation of tissue like immunology has attained both scientific and clinical significance and the well informed practitioner is expected to have a general understanding of the subject. This has been somewhat limited in the past because of technical words used by research workers to describe experimental procedures in transplantation Such special terminology serves to facilitate the exchange of information between pure scientists but it tends to exclude the clinician In this volume as in Volume I the material is presented in a simple direct manner with a minimal use of specialized terminology Research and clinical work are two sides of the same coin and should not function as separate entities Certainly the fully competent physician must have one foot in the laboratory and the other in clinical medicine the same may be said of research workers who like physicians tend to become narrow technicians in restricted fields

The second volume begins with an introductory chapter Cells and Tissues which comprises a review of Chapters 2 through 7 and Chapter 30 of Volume I with modifications based on recent work. This was included in Volume II on the advice of a number of professional friends who thoughtfully suggested that the rather simplified method of presentation would serve to orient the physician and surgeon for the chapters which follow and make him feel at home with tissue transplantation.

The editor takes pleasure in expressing a deep sense of appreciation to the contributors to this volume who have so ably presented their subjects in an understandable and concise manner These colleagues deserve credit for any success that the book may have Three chapters were written by associates in the Rehabilitation Department of Saint Barnabas Medical Center Others are by selected authorities, all of whom are well known in the field of transplantation

Once again I acknowledge my indebtedness to Dr George Lathrope and Dr Royce Paddock for encouraging me in my early transplantation studies of human tissues and to Dr William Bernhard Director of Laboratories at Saint Barnabas Medical Center for advice regarding microscopic interpretation of many tissue sections Dr Robert Ivy editor of *Plastic and Reconstructive Surgery* aided in various ways and an old friend Dr Clarence Straatsma, stimulated my efforts in completing this volume by his favorable comments in reviewing Volume I.

Dr George Schicks, as executive director of the Saint Barnabas Medical Center generously provided laboratory and hospital facilities for the experimental work

Miss Ruth Pullen R.N contributed drawings illustrating cell behavior in transplants and made valuable suggestions regarding them Miss Emma A Buehler M.A. compiled the literature and assisted in the editing of the volume

To my associate Dr John C Walker Jr., who aided in experimental investigations and to all of my former residents I am indebted for cooperation in carrying out portions of the work.

With deep regret the editor announces the demise of Dr Sterling Bunnell who contributed the chapter Transplantation of Nerves in this volume Dr Bunnell was the father of modern hand surgery and his death is a great loss to medicine

L. A P

# Contents

PART I

# *Cells and Tissues*
# *Zoologic Laws*

CHAPTER 1

# Cells and Tissues

LYNDON A. PEER

## THE CELL AND SMALLER LIVING UNITS

### Cells

Modern scientific thinking did not first produce the concept of very small units of life nor did it develop the idea of a cell theory. Early Greek philosophers, particularly Aristotle and Theophrastus, speculated that all animals and vegetables are constituted by a few elements which are repeated in each one of them. While they were largely concerned with substances that could be seen with the naked eye such as the roots, leaves, and trunks of trees and the tissues and organs of animals, the basic concept of large structures composed of and arising from smaller elements was established.

Robert Hooke (1), an Englishman, first described the cell as a unit of structure in 1665. Examining the texture of a cork through magnifying lenses, he observed that it contained many small compartments arranged in a honeycomb-like manner. Hooke noted the presence of limiting cell walls but gave little thought to "juice" or content of these cells.

The cell theory, which postulates that all plants and animals are composed of small units of life, was introduced by two German investigators, Schleiden and Schwann, in 1838 and 1839. They emphasized the significance of the jelly-like content of cells. Robert Brown, a botanist, in 1831 discovered the nucleus in cells. Purkinje in 1839 gave the name "protoplasm" to the basic substance of animal embryos, and seven years later von Mohl applied the same term to vegetable cells. Thus the gelatinous substance found in all cells became known by this name. These original investigations gave rise to the protoplasm theory, which states that the cell is an accumulation of a living substance—protoplasm—limited in space by a cell membrane and containing a nucleus. The cell is now accepted as the fundamental unit in the structure of both plants and animals just as the atom is accepted as the fundamental unit in chemical structures. Obviously the atoms in the molecules in the cell are made up of protons, neutrons, and electrons. This makes cells similar to inorganic matter with the important exception that a cell is *living* and inorganic matter is *dead*.

Recent studies utilizing polarization, diffraction, and ultramicroscopic and electron microscopic techniques have revealed many smaller complex structures in cells, bacteria, and rickettsiae. Microdissection and biochemical research has disclosed some of the possible functions and chemical composition of certain specialized particles found in the protoplasm. Thus the cell, which was formerly regarded as a simple structure, is now evaluated as an extremely complex center of diverse chemical activities.

Ultramicroscopy and electron microscopy now permit investigators to observe the "filterable viruses" that hitherto have been known only by their ability to pass through fine filters or by the pathologic alterations that they cause in human and plant tissue cells.

### Viruses

According to Stanley (2) there is no single criterion by means of which viruses can be differentiated from bacteria, but the virus has been segregated by means of certain general characteristics. Among the most important of these are its small size, the ability to reproduce or multi-

ply within the living cells of a given host, the power to change or mutate during multiplication and the property of reproducing or growing in artificial media containing susceptible host cells.

Viruses vary in size from 300 to 10 millimicrons. Certain small viruses are smaller than the accepted protein molecules and conversely some large viruses such as the vaccinia virus are larger than some of the smaller living organisms

In general the small viruses seem to have more primitive or simpler structures than the large viruses whose complexity of composition, structure, and function increases with their size. Because of this apparent direct relationship between the size of a virus and its complexity it has been suggested by Stanley (2) that the viruses provide a link between inorganic molecules and organisms and thus create an evolutionary pathway leading from simple elements such as the electron to massive, highly complex structures such as man.

The exact status of the virus has caused considerable controversy among biochemists and pathologists, some believing that it is viable or alive, and others that it is non-viable or inanimate. Some virologists believe that the smaller viruses, the crystalline ones in particular are non-living and represent end-products manufactured by their host cells through autocatalytic processes (3) Other virologists endow them with a sort of 'half life' between the living and the non-living state. Dogmatic beliefs regarding that which is living and, alternately, that which is non living have been subjected to some rather rude jolts in recent years, and for this reason precise distinction may be subject to later change.

An example of this difficulty in presenting exact evidence to demonstrate that *a structure is either living or dead may be seen in the tobacco mosaic virus This small entity in its extracellular state is a pure anhydrous crystal devoid of any water content whatever When the virus gets within a susceptible host cell, however it has the ability to reproduce and to change or mutate during multiplication thus resembling a living structure*

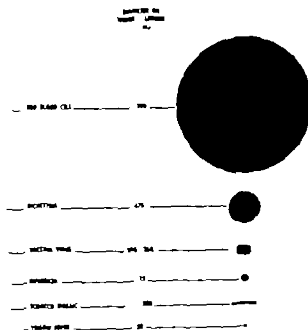


FIG 1 The size and form of various viruses compared to a human red blood cell Diameter or width × length in mμ (Modified drawing from T M Rivers Viral and Rickettsial Infections of Man J B Lippincott Company Philadelphia 1948 )

### Genes

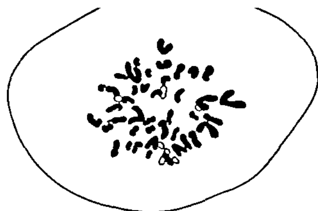
Direct chemical analysis of whole chromosomes demonstrates that they are largely composed of nucleoprotein (4) which suggests that the genes also probably contain a large amount of nucleoprotein. Thus a similarity in structure can be drawn between the gene and the more primitive small viruses which are also composed largely of nucleoprotein

Another similarity between genes and viruses is their power of self-duplication which is dependent upon the presence of certain substances found in living cells. Both genes and viruses multiply only within specific cells where certain necessary substances are available and the environment is satisfactory One important difference, however is the fact that genes are found only within a living cell, whereas the virus can exist extracellularly (in a chemical sense)

Genes and viruses are also within the same general size range (5) and both can undergo mutation giving rise to new forms which have altered biologic characteristics. These new forms moreover retain their power of self-duplication (5)

### Rickettsiae and Bacteria

A number of human infections are caused by microorganisms called 'rickettsiae. These are intermediate in size and other characteristics between bacteria and viruses. Studies with the electron microscope reveal the structure of rickettsiae to consist of an apparent limiting



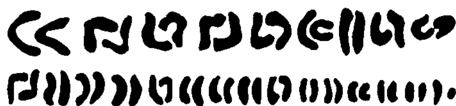


FIG 2. *Above* The chromosomes in a human cell ×3000 (Koller) *Below* The twenty four pairs of human chromosomes a drawing based upon thirteen male nuclei (From Evans and Swezy's *Chromosomes of Man* ) These drawings show the chromosomes as relatively short thick bodies which are characteristic of the stage just preceding cell-division during which they can be counted and their individual appearance discerned At an earlier stage they are long thin filaments (From J A Fraser Roberts An Introduction to Medical Genetics p 2 Humphrey Milford Oxford University Press London 1940 )

membrane surrounding a protoplasm-like substance which contains a number of dense granules. A distinct and recognizable nucleus has not been observed. The rickettsiae are visible in microscopic preparations as coccobacillary forms and, like viruses they multiply only within susceptible cells

Bacteria were formerly thought to be the simplest and lowest of all living forms beyond which life did not exist. Exmination in previous years with the ordinary compound microscope failed to reveal a nucleus or any other structure within the substance of bacteria. Examination with the electron microscope and special staining reagents, however has demonstrated the presence of bodies within bacteria that apparently are equivalent to the vesicular nucleus in typical cells (6) Hence a bacterium is now called a bacterial cell. Some botanists regard the bacteria as plants which like fungi, do not contain chlorophyll others believe that they are intermediate forms between plant and animal.

## THE HUMAN TISSUE CELL

The findings of Schleiden concerning the constitution of living matter in plants were confirmed in and extended to animals by Schwann who for the first time, used the term cell theory for the concept that animals as well as plants are aggregates of cells arranged in accordance with definite laws. The cell theory was then rather quickly applied to explain the structure of unicellular organisms spermatozoa, and that of the ovum from which—by division of cells—the organism is developed

The main difference between plant and animal cells is that the former contain chlorophyll which is so vitally sustaining that the food it synthesizes supports all organic life on the earth. Certain plants such as the fungi do not contain chlorophyll, but those which do not have it must steal its products from other plants in order to exist.

The cell principle includes two concepts 1) that the bodies of all plants and animals are composed of cells and the products of cells and 2) that new cells are derived only by the division of preexisting cells. Speculating on how the primitive ancestral types from which all modern plants have been derived were first brought into existence, biologists have come to favor certain hypotheses The most plausible one seems to be that the first living organisms upon earth were derived

from non-living materials already present but just how this occurred remains a mystery, for man has not been able to produce life from non-living materials or to discover anything of the sort occurring spontaneously in nature today

## Cell Division

The cell perpetuates itself by cell division which is a universal activity in our world. Through the process of cell division the chromosome splits lengthwise, half moving toward one pole and half toward the other A new cell wall cuts the two groups across the middle and the two halves separate forming two cells. In this way every cell is part of a series, or a unit in the sequence of reproduction which is a sort of immortality *

Growth of the human individual, as is well known is effected by consecutive cell division called mitosis. At first this growth is rapid, the germ cell dividing into two daughter cells which in turn will produce a generation of 4 then 8 16 32 and so on. The human tissue cell is estimated to be only about the fiftieth or sixtieth descendant of the egg from which the human being evolves.

## Plasma or Cell Membrane

The cell membrane is a complicated and important structure which controls the entrance and exit of all substances necessary for cell metabolism. The cell membrane is so thin that it is beyond the limits of microscopic visibility with ordinary light it is visualized as the surface of separation between the cell content and the tissue fluid outside the cell. Special studies indicate that the cell membrane consists of a continuous layer of lipoid molecules arranged radially to give permeability with absorbed layers of protein molecules arranged tangentially to provide tensile strength

The ionic content of cells, which may differ from that of the surrounding fluid medium is maintained throughout the life of the cell by continuous control by the cell membrane of the entrance and exit of molecules and ions. The cell membrane is permeable to both water and some solutes, but the passage of various other solutes through the membrane does not occur with the same facility In general, osmotic pressure is preserved by a mechanism which regulates the concentration of dissolved substances within the cells. In human beings the osmotic pressure as a whole is regulated mainly by the kidneys and sweat glands.

## Fluid Environment of Cells

The human tissue cell is essentially an aquatic entity in this respect resembling the single-celled structures living in the ocean and in fresh water ponds. Both absorb through their cell membranes from the surrounding liquid medium the materials which they require, and excrete similarly through the membranes to the medium waste products which they desire to be rid of Most of the several billions of living cells constituting the human individual are surrounded by interstitial fluid, and living cells on the body surface in contact with air are protected by moist films as in the eye nose, and mouth. Surfaces not covered by moist films are protected by layers of dead cells as in the epidermis, which prevents the living cells from drying out.

In the excretory processes of gland cells in the pancreas and thyroid and in the elimination of collagenous material by the fibroblast as described by Stearns (7) excretion is accomplished by a pinching off of the plasma membrane containing the secretion, somewhat similar to a minor cell division. The point where the surrounded cytoplasm pinches off remains covered by plasma membrane.

## Cell Content

The plasma or cell membrane both surrounds and contains the jelly-like protoplasm. This protoplasm is composed of two structures, the cytoplasm and the nucleus, which are the sites of internal specialization occurring in cells. Modern studies have demonstrated the constant presence of the nucleus, or its equivalent, in every living cell and its important role in cell activities. The nucleus appears to be concerned mainly with growth the reproductive cycle, and the imparting of character The cytoplasm controls simpler cell activities such as secretion and excretion phagocytosis, absorption and contractility Among certain basic concepts regarding the structures in the cytoplasm and nucleus is the fact that the nuclear membrane, through which substances going to and from the cytoplasm

* It has been generally accepted for some time that all human tissue cells contain exactly 48 chromosomes Recent experimental work however has indicated that this may not be so

must pass is important for the survival reproduction, and functioning of the human tissue cell.

Very little is known regarding the molecular composition of nuclear membranes, but they must be rather tough since intact nuclei can be centrifuged and collected after rupture of the cell membrane and removal of the cytoplasm. Cytoplasm has the ability of healing itself after moderate-sized rupture in the presence of the calcium++ ion. This property is not possessed by the nucleus: when the nuclear membrane is broken, its content flows out and the nucleus collapses without showing any tendency to repair (8).

The interrelationship between the nucleus and the cytoplasm is necessary for the activity and life of a human tissue cell. Experiments with fragments of cells without a nucleus have demonstrated that the denucleated cytoplasm survives only a short period of time and is not able to grow or reproduce. Nuclei themselves cannot live as isolated entities since they require a certain quantity of cytoplasm in order to be maintained (9).

### Form and Size of Cells

Human tissue cells vary in size and shape, depending on the type and also somewhat on their location and phase of activity. Cells living freely in their fluid medium, as in the blood or lymph, and not closely packed together often have rounded contours. An exception is the red blood cells, which are non-living bags of oxygen-carrying hemoglobin. Cells that are closely packed together, such as epithelial cells, are roughly hexagonal in shape.

Although different types of cells vary as to size and shape, there appears to be a general *uniformity* among those of similar types. *Striated* or voluntary muscle cells, which are multinucleated, are among the largest cells in the body and some lymphocytes are among the smallest cells; nerve cells are the longest—but so thin that they are quite invisible.

## DIFFERENTIATION, AGING, AND DEATH OF TISSUE CELLS

The higher organisms develop from a single cell—the fertilized ovum—which in the human being gives rise through fifty or sixty successive divisions to all structures in the body. At first













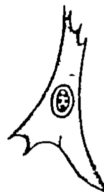



FIG. 3. Drawings illustrate variety in size and shape of tissue cells.

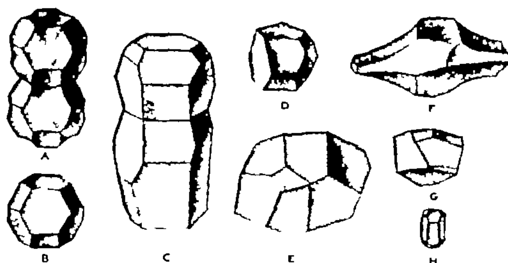



FIG 4 Models of ideal and actual cells A Two of Kelvin's 'minimal tetrakaidecahedra' which solve the problem of dividing space into uniform bodies of minimal surface B Kelvin's orthic 14-hedron with a slightly greater surface area than the minimal all facets are plane and regular C–H Reconstructions of actual cells C Two elder pith cells *above* a 13-hedron *below* an irregular 14 hedron D An 11 hedral cell of elder pith E Human fat cell 16-hedral with many pentagonal facets and an atypical *tetrahedral angle H G and F Basal middle and outer cells from the stratified buccal epithelium of* human embryo except at the basal and free surfaces of the epithelium the enlarging cells maintain an average of 14 facets basal cells have a single facet toward the underlying tissue—11 facets altogether Approximate magnifications C ×170 D ×150 E ×300 F–H ×750 (F T Lewis) (From R. O Greep Histology p 21 The Blakiston Company New York 1954

the process is a simple increase in the number of cells but in man after the blastula has been formed, the cell divisions become qualitative

**Changes in Differentiation**

In this process of differentiation the nucleus, which plays such an important part in heredity changes very little. The cytoplasm, however may differentiate greatly giving rise to the fibrillae in muscle cells which have the property of contraction the axons of nerves which represent a sort of cytoplasmic extension or tail the ingestion of fat, resulting in the swollen fat cell and so forth. The cytoplasm also plays a dominant role in producing the inanimate intercellular substance, giving rise to or associating in the production of such different dead substances as are present in bone tendon, and cartilage which have large amounts of intercellular substance and relatively few parenchymal cells. Thus, most directly through activity of the cytoplasm the cells are sorted out into the three germ layers—the ectoderm, mesoderm, and endoderm—which through further differentiation form the various tissues and organs in the human body

The life expectancy or normal life span of the different human tissue cells varies greatly For instance after development in early infancy no new peripheral nerve cells are produced. This is true also of heart muscle and possibly skeletal muscle The red blood cells, however have a chemical life span of about three months (more or less) and are constantly being replaced by nucleated cells from the bone marrow The cells in the basal or germinal layer of the epidermis as well as those in the more superficial stratum lucidum are continuously undergoing cell division throughout an individual's life in this manner they produce the horny surface layer of dead cells which retain the aqueous medium surrounding the deeper living cells. The life span of cartilage cells after they stop dividing is not known This also applies to fat cells, tendon and fascia cells bone cells and others.

One should bear in mind that apparent growth of such structures as cartilage, tendon, fascia, and other tissues may take place by an increase in the intercellular substance without increase in the number of cells through cell division.

The growth of cells may be by means of either enlargement or *multiplication. Cell division* is necessary for continued growth for otherwise the cell would soon reach a size where its surface would be inadequate (for nutritive respiratory

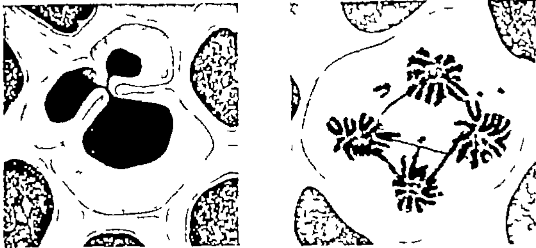


FIG 5 Abnormal nuclear responses in dying cells Corneal cells of a urodele larva exposed to x rays *Left* Asymmetrical tripolar amitosis *Right* Cell with four nuclear masses connected by chromosome bridges (Alberti and Politzer) (From R O Greep Histology p 29 The Blakiston Company New York 1954 )

and excretory purposes) to its mass. In general, however cell division is most active in the early embryonic periods during which the cells remain small. Later division diminishes or ceases in some parts of the body, and growth is due chiefly to the enlargement of cells already present. The growth of the structural units of organs also follows this general rule, the production of new units being confined mainly to fetal and early postnatal life (10)

### The Aging Process and Death of Tissue Cells

After differentiation occurs almost all cells pass on to a phase of aging or senescence which ends in death The concept of an elixir of life to produce longevity has long served as a stimulus for mankind Although the elixir has not materialized attempts to discover wherein lies the secret of the prolongation of cellular life have been made

Evidence indicates that the length of life may be determined by the structure of the chromosomes, since animals can be bred with longevity as a dominant. There are also many human families in which longevity commonly occurs in successive generations. It seems probable therefore that the life span of the cells which undergo senescence is partly determined by heredity

Throughout the individual s life many cells are aging and dying, as illustrated by the constant death and replacement of red blood cells and by the disintegration of the external horny layers of the epidermis. In general those cells which retain their capacity to divide continuously do not age. Thus mitosis appears to be a constant rejuvenizing action which retards the process of senescence.

Senescence appears to be caused partly by changes in the fluid medium in which cells live for most cells this means the interstitial fluid rather than the blood Studies by Carrel (11) have demonstrated that blood plasma contains both growth-promoting substances and inhibitory substances. The latter increase with age and reach a high concentration in old age These inhibitory substances may be a factor in the causation of senescence.

Krohn (12) demonstrated recently that *old ovaries grafted into young animals* behave more satisfactorily according to the data so far presented on the estrous cycles than *young ovaries grafted into old animals*. This would confirm the

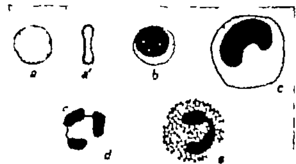



FIG 6 Blood corpuscles *a* Red blood corpuscle seen in surface view and *a* in profile *b* Small lymphocyte *c* Monocyte *d* Polymorphonuclear leukocyte *e* Eosinophil leukocyte ×1120 approx (From W E Le Gros Clark The Tissues of the Body ed 2 p 210 The Clarendon Press Oxford 1945 )

general impression that ovarian function in rodents is related to pituitary activity and not to changes inherent in the ovaries themselves. Thus it is a far cry from the days of Veronoff and the expectation that *transplants of gonadal tissue* would serve to rejuvenate the senescence. It is now evident that such hopes, which rested on the possible benefits of heterotransplantation, were bound to be illusory

According to Parkes (13 14) the successful preservation of cells tissues, and organs at low temperatures offers immediate possibilities in the study of the comparative aging of different parts of the body It opens up the ultimate possibility of inducing suspended animation of the whole body for indefinite periods. The idea of biostasis of the whole body although somewhat macabre holds nothing new in principles being woven into the fabric of countless human beliefs legends and stories from the resurrection of the dead to the awakening of the Sleeping Beauty

The successful work with organs and tissues turned attention to the problem of cooling the whole animal to such an extent as to arrest all vital processes and confer upon it, in a state of suspended animation, the kind of comparative immortality which can be effected with spermatozoa from the bull and other cells appropriately frozen and maintained at −70°C An approach to this problem has been made possible by the work carried out by Andjus (15) who evolved a method by which rats could be cooled to deep body temperatures approaching 0°C and revived after cessation of heart beat and respiration for one or two hours. Later work demonstrated that deep body temperatures as low as −5°C could be obtained without crystallization and the animal resuscitated completely (16)

## Chemical Reactions

The cell resembles a small chemical factory which is equipped to accomplish synthesis and breakdown of various substances at the level of body temperature. Chemists can carry out many of these same reactions in the laboratory but

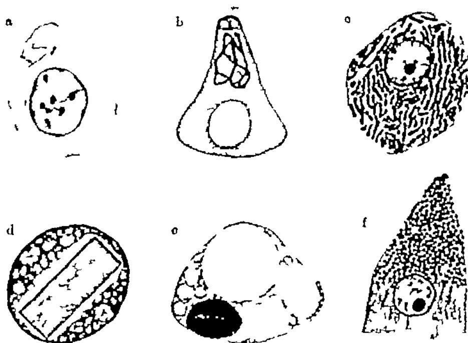



FIG 7 Various cytoplasmic organelles and cell inclusions Because of their specific physical and chemical properties these objects are not generally demonstrated by routine methods and are rarely revealed together *a* Centrosome in cell of grasshopper testis showing paired centrioles (diplosome) and radiating aster *b* Golgi material in *pancreas of cell guinea pig* as demonstrated with osmic acid fixation (Redrawn from Cowdry Special Cytology ) *c* Mitochondria in a liver cell of a dog stained with hematoxylin (Weatherford) *d* Crystal within the nucleus of a liver cell of a dog (Weatherford) *e* Spaces left by dissolved fat young fat cell *f* Secretory granules in a pancreas cell human Below and around the nucleus lies the cytoplasmic basophilic material (basal ergastoplasm) (*From R O* Greep Histology p 17 The Blakiston Company New York 1954 )

under conditions of high pressures and high temperatures which are not present in the human body The tissue cell effects these chemical reactions through the activity of enzymes, substances capable of producing such reactions at body temperature

It is now understood that the presence of certain trace substances is necessary for the building up of enzymes which are constantly being destroyed by the chemical reaction in which they are involved. The presence of certain vitamins is essential for the effectiveness of the enzymes (the enzyme-vitamin complex) and hormones in turn play an important part in regulation and control

Essential cell activity may not only be interrupted by the absence or deficiency of the substances enumerated above but it may also be adversely affected when they are present in excessive amounts Certain substances therefore are produced by the body to serve as inhibitors or neutralizers so that a normal rhythm of activity in the factory cell may continue. Alteration in any essential link of this vital chain may cause the living tissue cell to age and eventually die just as surely as taking away its raw materials—the food substances— which the enzymes, vitamins and hormones affect

## INTERCELLULAR DEAD SUBSTANCES SURROUNDING TISSUE CELLS

The process of cell division initiated by the fertilized ovum is a simple increase in the number of cells until the blastula has been formed. A group of cells known as the organizer which is located in the dorsal lip of the blastopore, then assumes control of the cell division and causes a grouping of cells into the three germ layers—ectoderm mesoderm and endoderm (17)

The formation of three germ layers represents a sorting out of tissue cells to provide a proper and efficient division of labor for building the human body and to provide for its future servicing and maintenance.

The nuclei of cells in the three primary germ layers undergo little change but the cytoplasm

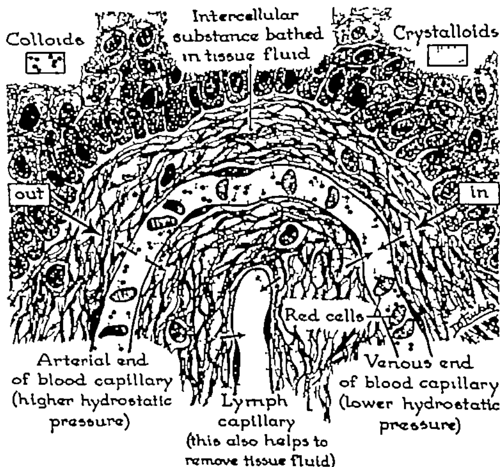



FIG 8 Intercellular fluid from the capillary bed maintains the fluid environment of every tissue cell It returns to the closed vascular system through the venules and through the lymphatics (From A W Ham Histology p 93 J B Lippincott Company Philadelphia 1950 )

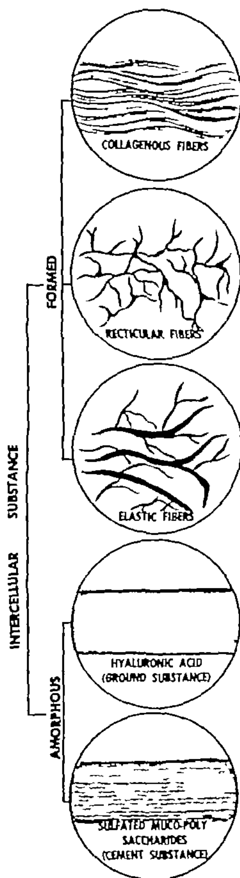



FIG 9 Nonliving matrix between cells (Modified drawing after A. W Ham Histology J B Lippincott Company Philadelphia, 1950 )

differentiates and becomes specialized to such an extent that the mature progeny have little resemblance to the original. Regardless of these differences due to changes in the cytoplasm the cells retain their basic similarity all have a nucleus contained in the nuclear membrane and a plasma or cell membrane surrounding the specialized cytoplasm.

The non-living intercellular substance surrounding cells is believed to be produced and maintained by the activity of the living cells or in association with them. This activity may occur as a budding off of cytoplasm to form intercellular collagenous fibers as in the construction of a tendon, or the cell may appear merely to stimulate and maintain the building operation as in bone.

## Organization of Cells

The cells in the three primary germ layers undergo further differentiation, resulting eventually in highly specialized cells with definite functions. Thus, cartilage cells produce cartilage matrix bone cell elements influence the deposition of calcified intercellular substance in which they are later surrounded tendon and fascia cells throw off cytoplasmic buds which form collagenous fibers epidermal cells, with little intercellular substance, reproduce endlessly to provide a protective horny layer on the body surface.

Some cells retain their embryonal or more primitive characteristics, and it is believed that these cells can do a number of different things which are essential for the servicing and maintenance of the body when the occasion arises.

## Specialization of Tissue

Thus, through specialization in the cytoplasm of cells and by the elaboration or controlled deposition of different intercellular substances, various tissues and organs are constructed. In muscle, fatty tissue, and nerve tissue, alterations in the cytoplasm are important factors, whereas in bone cartilage, and tendon the dead intercellular substance dominates the scene and provides important qualities for these tissues.

The development of specialized tissues from the ectoderm mesoderm, and endoderm is not, however a mere matter of cell division and cell differentiation. As pointed out by Medawar (18) "the tactics of embryonic development and of

regeneration are a matter of movement of cell substances, cells and cell groups.

During the orderly development of highly specialized human tissues from a mass of undifferentiated cells, "a division of labor" occurs among cells, some having one task and others having another. In this way there arise specialized tissues and organs made up of many different kinds of cells which have considerable variation in size, shape, and function.

### Intercellular Substances

Intercellular substances, which may be *fibrous or amorphous gels*, are the dead parts between cells. They serve to hold the body together in various ways, providing rigid strength as in calcified bone, elasticity as in the gelatinous matrix of cartilage or support the cells in a loose and movable mesh in other areas.

Since intercellular substances are usually interposed between capillaries and the cells which they nourish, all, regardless of their apparent density must permit diffusion of substances from capillaries to cells and vice versa. Amorphous intercellular substances such as gels or sols usually permit better diffusion than fibrous substances. Fibrous intercellular substances consist of collagenous, reticular and elastic fibers; the amorphous gels occupy the spaces between them.

The *amorphous intercellular material* consists of cement substances (sulfated mucopolysaccharides) and a ground substance (hyaluronic acid). Hyaluronic acid has a cohesive quality which holds the tissue cells together. An enzyme, hyaluronidase, reduces the viscosity of hyaluronic acid and, in separating its molecules, produces the spreading factor that allows the cells to separate farther apart; this increases the size of the intercellular spaces.

The origin of the ground substance is obscure, its turnover, if any, is not known and its function is still a matter of speculation (19). In the adult, ground substance may be produced by mast cells from a heparin-like precursor, or it may be secreted or formed due to the activity of fibroblasts. According to some authorities ground substance may, in a reversible way, vary from a

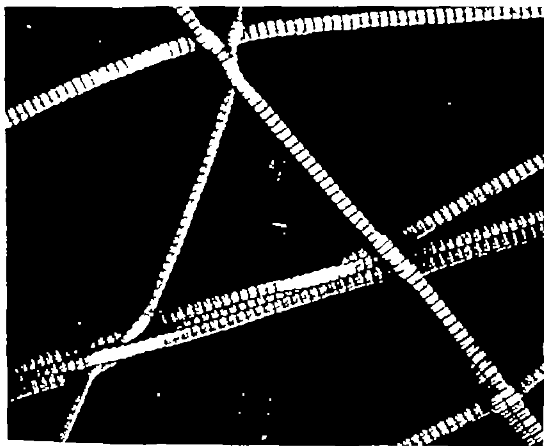


FIG 10. Electron micrograph of collagen fibrils from human skin. Notice the characteristic cross banding of the fibrils at intervals of 640 Å along their length. ×32,000. (Courtesy of Drs. Jerome Gross and F. O. Schmitt, Dept. of Biology, Massachusetts Institute of Technology.) (From R. O. Greep: Histology, p. 87. The Blakiston Company, New York, 1954.)

more or less fluid state to a rigid gel such as the matrix of cartilage. Whatever the physical state of the ground substance it must permit a two-way exchange of substances between the closed vascular system and each individual tissue cell.

*Collagenous fibers* are now believed to be produced by the fibroblast as a sort of budding off process from the cell. *Reticular fibers* are immature collagenous fibers, and there is a gradual transformation of reticulum into collagen in the process of aging.

The origin of *elastic fibers* is still a controversial issue: they appear to be constructed from the alignment of refractile granules laid down in the

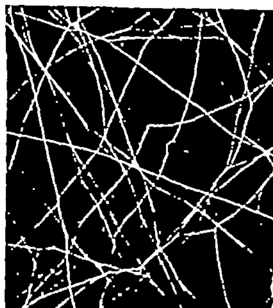


Fig 12 Network of elastic fibers in the rat mesentery. The preparation was stained with resorcin fuchsin and the photomicrograph printed as a negative ×450 (From R. O Greep Histology p 86 The Blakiston Company New York 1954.)

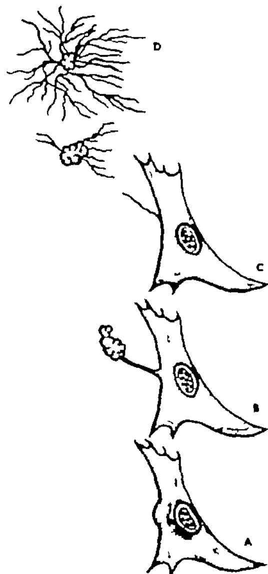



Fig 11 Diagram showing how collagenous fibers are formed by fibroblasts. (Redrawn after Clark.) (From a paper by M L Stearns Am J Anat 67 55 1940.)

intercellular matrix by some unknown agency. There is little evidence that new elastic fibers can be formed when they have been destroyed, and it seems probable that the original fibers last through the individual's lifetime. In senile elastosis the normal bed of delicate elastic fibers in the dermis is replaced by a coarse mat of large elastic fibers according to some investigators. Other authorities, however, believe that the elastic staining material in senile elastosis is not elastic tissue. It is altered collagen—considerably altered but still collagen.

### Organs and Endocrine Glands

An organ is considered as any part of the body consisting of more than one tissue and serving a definite function or functions. The heart, lungs, and kidneys are obviously organs, but difficulties are encountered when we try to catalogue the status of simpler structures (20). An artery is clearly an organ but a capillary consisting of only one tissue, endothelium, does not merit the term. A bone is an organ, but the cartilaginous structure of the chest cage consists of a single tissue which is devoid of blood vessels, nerves, and lymphatics. One therefore hesitates to list this cartilage as an organ despite its important functions.

A number of organs such as the pancreas and ovary serve also as endocrine glands, and it is difficult to define an endocrine gland without becoming dogmatic. Cowdry (20) notes that all living cells give off materials into the surrounding medium which influence their neighbors and other cells that are far distant. Although the word hormone (Gr *hormao* excite) was introduced with the idea that the substances are activators it has been found that some of them are inhibitors. Yet the inhibitors in common with the activators are called hormones All hormones are at any rate *chemical messengers* which travel in the blood stream *Endocrine glands may be properly considered as endocrine organs*

## STRUCTURE AND VIABILITY OF CELLS

### Tissue Culture

The most difficult and frustrating problem in evaluating the behavior of free tissue grafts is to determine accurately whether the cells have remained viable or are dead structures, which take the various dyes and demonstrate normal appearing cell architecture.

One of the methods which permits us to observe the cells not only in a state of mere survival but under more favorable conditions and also to follow their development is *tissue culture* Small portions of various tissues are transplanted in a suitable medium where the cells can adapt themselves and grow in an autonomous form. In growth the cells spread over the coagulum of plasma and pass out from the transplant to form a "zone of growth" which permits vital observation of these cells. Undoubtedly this would be the most accurate and final way to determine whether the graft cells have survived or have failed to survive, provided a tissue growth is obtained. If growth does not occur the possibility still would remain that the graft cells were viable but were not capable of growth in the media. Different adult human tissues vary in their ability to grow in tissue culture.

### Fixation and Staining of Cells

In addition to the structures that are observed in vivo which are more or less modified by fixation other structures are found by staining which were not apparent before, due to the similarity of their index of refraction with that of the rest of the cell.

Fixation is essentially a method of preserving the morphology and chemical composition of the cell (21) The object of the fixation is to bring about the death of the cell in such a manner that the structure which the living cell possesses is conserved with the minimum of artifacts. Some methods, at the same time, attempt to maintain the chemical composition of the cell as intact as possible. The rapidity with which the fixative penetrates does not appear to depend so much on its coefficient of diffusibility as on the protein barrier impeding further passage of the fixative.

Fixatives, however produce currents in cells and these may displace the soluble components. Besides displacing the soluble substances fixatives extract with greater or less intensity Thus electrolytes, soluble carbohydrates and even lipids may leave the cells by the action of the fixatives. Fixation may additionally produce considerable chemical modification.

Fixation and later treatment produce a shrinkage of the tissue and this is important in interpreting the cytological images in fixed tissues. *One should always remember that the volume of fixed cells is less than that which they had in the living state*

Beside the examination of living cells the examination of fixed and stained cells is essential to a better understanding of cellular structure. The two methods are complementary and one does not exclude the other

### Fixation by Freezing and Drying

A technique devised by Altmann* (22) and brought to practical use by Gersh (23) permits us to investigate the structure and distribution of chemical components with a minimum of modifications.

Drying can be effected by reducing the partial pressure of water vapor in the atmosphere surrounding the frozen tissue. The water in the frozen tissues passes off directly into a gaseous state, producing a progressive dehydration but no shrinkage of the tissue. The chemical composition is maintained practically without change, and the structure is preserved with very few modifications produced by ice crystals. The rapidity of fixation permits one to trap and preserve cells at critical moments of their function.

* Altmann discovered mitochondria in the cytoplasm of cells and believed that they were living particles

The freezing-drying technique of Altmann-Gersh should be considered as an intermediary between the examination of fresh and fixed tissues

### Vital and Supravital Dyes

According to Ham (24) both vital and supravital staining depend on the interaction of vital activity and a dye. *Neither vital nor supravital stains will produce their characteristic effects if they are applied to dead cells*"

*Vital staining* is accomplished by injecting usually intravenously into living animals certain dyes of a colloidal nature (trypan blue) or certain metals in a colloidal state (colloidal silver) or fine particulate matter (suspension of fine carbon particles as India ink) The cells of the reticulo-endothelial system being phagocytic possess the ability to incorporate the colloidal particles of the vital stain into their cytoplasm, and become colored by the dye particles which they accumulate.

*Supravital staining* (24) is generally accomplished by 1) injecting dyes into the blood vessels of animals immediately after they have been killed and while the cells to be stained are still surviving or 2) applying dyes to freshly removed pieces of tissue in which the cells are still alive.

Dilute solutions of supravital dyes have been used to stain the cells in fresh shavings of autogenous human cartilage grafts by the author's group The supravital dyes have also been applied to fresh control cartilage shavings and to preserved cartilage with dead cells. The observations made on a large number of autogenous grafts in this manner indicate that *the cartilage cells in human autografts survive transplantation as living chondrocytes*. Keloids may also be examined in the fresh state to some extent. With this method there is a complete absence of shrinkage, and the chemical composition is maintained practically without change.

One may also apply dilute solutions of supravital dyes to fresh sections of a homogenous human cartilage graft and observe that *the chondrocytes take the dyes as living cells* thus indicating that they have survived and are viable.

### Transparent Windows

The transparent chamber technique devised by Sandison (25) was modified by Algire and Legallais (26) for the study of tumor transplants and was first applied to a study of autogenous and homogenous skin grafts in mice by Herbert Conway and his associates (27) in 1951 Doyle Joslin (28) in 1952 further modified the transparent chamber as devised by Algire and Legallais. Transparent windows which can be inserted by surgical procedure in some part of the body afford clear observations of living tissue cells. It is a valuable technique for the study of the behavior of tissues, and its use will undoubtedly be extended to all types of free transplants. The method of approach however has certain deficiencies and again demonstrates the necessity for the use of all available methods of investigation, since all of them together are often insufficient to establish definite facts which cannot be questioned.

### The Phase Microscope

The light microscope has been improved by the development of phase-contrast microscopy Many of the minute constituents of tissue are quite similar in respect to optical density For this reason they transmit and refract light almost equally and when fresh unstained tissue is examined with the ordinary microscope, the particles do not stand out in contrast with one another The phase-contrast microscope permits the minute structures which differ from one another in optical density only slightly to be brought into sufficient contrast to permit their successful study in fresh unstained tissues

The electron microscope dissection of cells under the microscope by manipulating instruments, and the ultracentrifuge are additional agencies which are of great value in determining the structure, chemical composition, and physiology of cells. None of these techniques has as yet been applied to human tissue grafts.

### The Dissecting Microscope

Taylor and Lehrfeld (29) have examined skin autografts and homografts *in situ* in rats, mice, and rabbits through the dissecting microscope. They noted that the circulation of blood within the capillaries of grafts is similar in both autografts and homografts and that the time of breakdown of circulation in skin homografts (at about the seventh or eighth day) is consistent in rats mice and rabbits.

The author's group at the St Barnabas Rehabilitation Center have not observed that

the time of breakdown of circulation in human skin homografts is as consistent as Taylor and Lehrfeld reported in rats mice and rabbits. Human homogenous skin transfers between parents and infants may be tolerated for much longer intervals before eventual rejection. Furthermore when gross signs of rejection begin, the phenomenon of rejection is not necessarily progressive and irreversible. Some of the author's human skin homografts developed central necrosis at about the tenth day but completely recovered and enjoyed a long survival time in a series of skin exchanges between mothers and their infants or older children (30)

## THE PARENCHYMAL CELL IN *AUTOGENOUS GRAFTS*

The parenchymal cell is the essential or specialized part of a tissue or organ as distinguished from the supporting connective tissue. It would be well for every surgeon to have a clear conception of the parenchymal cell and of the factors which affect its survival in manipulated and transplanted tissues, because the viability or *death of this cell often determines the fate of the* tissues.

Experimental work and clinical observation indicate that survival of the parenchymal cells is associated with retention of the particular tissue structure in living autogenous grafts of cartilage fascia tendon, fat, skin certain bone grafts, and possibly in the interstitial portion of corneal homografts.

### Matrix

The living parenchymal cell is not only responsible for or associated with the *retention of an intercellular substance* it also appears to determine generally that this dead matrix *remains as the same kind of intercellular substance* in six types of autogenous tissue grafts. In this manner the matrix of hyaline cartilage remains hyalin the matrix of elastic ear cartilage remains as elastic substance the collagenous fibrous matrix of tendon fascia and dermis remain of the same character as do the small amount of intercellular material surrounding grafted epidermal and fat cells and the calcified matrix of certain bone grafts in soft tissue sites

Free autogenous human grafts in which the parenchymal cells have been intentionally killed before transfer do not usually retain their normal matrix structure. Thus autogenous cartilage grafts with dead cells tend to be gradually absorbed and replaced by fibrous connective tissue or bone autogenous tendon and fascia grafts with dead fibroblasts do not retain their same structure. Autogenous septal bone grafts with dead cells in soft tissue sites are gradually absorbed whereas *similar grafts with living cells retain their calcified matrix in soft tissue sites*

Dead autogenous tissue grafts with large amounts of non-living intercellular substance (cartilage bone, fascia, and tendon) tend to be absorbed more slowly than dead autogenous grafts composed largely of cellular elements (epidermis fat muscle)

The gelatinous intercellular substance of cartilage is the most durable of *all* intercellular substances with the possible exception of elastin. Following transplantation, cartilage matrix may even persist for long periods of time as an autogenous graft when the cells in the graft have been previously killed by heat or other agencies.

### Identification of Types of Parenchymal Cells

It is not difficult to select the parenchymal cell of *cartilage* for the tissue contains only one cell which is surrounded by its own intercellular matrix. The ultimate fate of a free autogenous cartilage graft appears to depend largely upon the survival of the chondrocytes which are the only living things in the graft. If the cells in a cartilage graft survive transplantation, they will *as a rule* continue to service and maintain their intercellular matrix, so that the bulk of the graft and the specific structure of the intercellular substance will *usually* remain the same. If the *cells fail to survive* or are *killed* by heat before transplantation the bulk of the graft tends to be slowly reduced and replaced by fibrous tissue or its derivatives.

The parenchymal cells in *tendon* and *fascia* are also easy to identify because these tissues resemble cartilage in having a single cell type supported and surrounded by intercellular material. This intercellular substance in tendon and fascia is composed largely of collagenous fibers. The intercellular substance of autogenous fascia grafts appears to be retained *when the* fibroblast cell, which represents the essential or parenchymal cell survives The collagenous bundles in tendon also appear to be retained

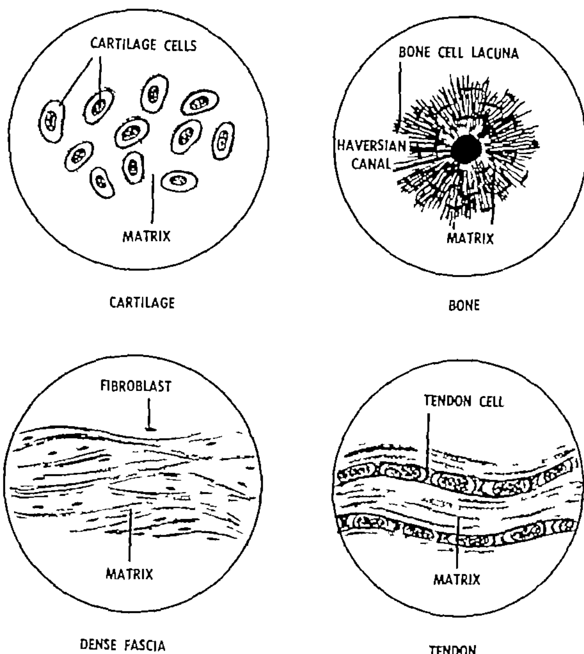



Fig 13 Cartilage bone dense fascia and tendon These tissues have similar basic structures in that they are composed of only one type of parenchymal cell which is surrounded by a large amount of non living intercellular material or matrix This inanimate intercellular substance which is the product of activity of the cells determines the specific structure of these tissues Thus cartilage is firm but relatively elastic due to a gelatinous intercellular substance bone is rigid due to a calcified material between its cells and fascia and tendon are pliable and strong owing to the presence of tough bundles of fibers between the cells

All of these tissues are derived from mesoderm and hence their respective parenchymal cells are descendants of primitive mesenchymal cells which have become specialized cartilage cells bone cells fascia cells or tendon cells All excepting cartilage contain blood vessels and nerves

when the parenchymal tendon cells and stromal fibroblast cells survive transplantation.

When the tendon and fascia cells fail to survive, the fate of the collagenous fibers is not definitely known If the graft is in contact with unlike tissues the fibers may disappear When it is in contact with like tissue it may be possible for the tendon and fascia to be replaced by creeping substitution as in dead bone grafts in contact with living bone.

The bone cell is generally acknowledged to be the parenchymal cell in *bone* with acceptance

FIG 14 Cross section of second phalanx of a human middle finger showing replacement of spongy bone by compact bone ×110 (After Schaffer ) (From A A Maximow and W Bloom A Textbook of Histology ed 6 p 120 W B Saunders Company Philadelphia 1952 )

of the osteoblasts (or some other agency) as the builders of this tissue. The bone cell has the ability to retain its calcified matrix in certain types of bone grafts, with a complete absence of osteoblasts and osteoclasts, provided the osteocytes or parenchymal bone cells are viable.

Bone grafts must be roughly divided into two types those which have very little regenerative power (usually membranous bones) and other types which have regenerative power Grafts of bone without regenerative power include nasal septal bone the nasal bones, and the turbinates these tend to retain their calcified matrix regardless of whether they are transplanted in contact with bone or with soft tissue, provided the parenchymal cells survive. When the parenchymal cells in these autogenous bone grafts are killed by heat, desiccation or other agencies before transplantation in soft tissues the calcified intercellular matrix is slowly absorbed

Other types of bone, such as rib tibia, and iliac

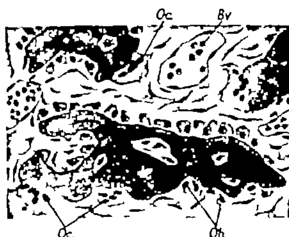
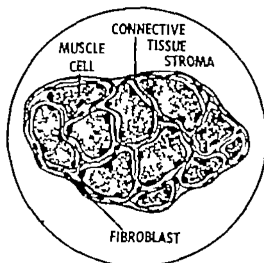
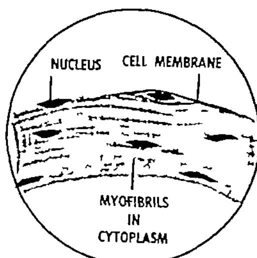


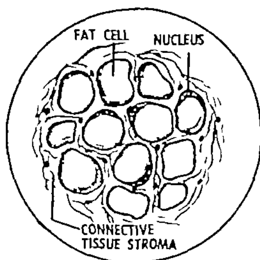

FIG 15 A drawing (semi-diagrammatic) showing osteoblasts (*Ob* ) and osteoclasts (*Oc* ) in ossifying bone Note that some of the osteoblasts become enclosed in newly deposited bone to form bone cells or osteocytes The tissue is richly vascular and some thin walled blood vessels (*Bv* ) can be seen in section (From W E Le Gros Clark The Tissues of the Body ed 2 p 74 The Clarendon Press Oxford 1945 )



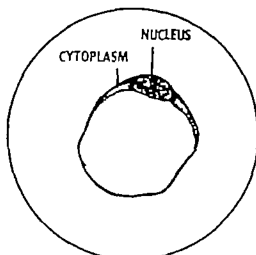

MUSCLE BUNDLE




MUSCLE CELL




LOBULE OF ADIPOSE TISSUE




FAT CELL

FIG 16 Muscle and adipose tissue These tissues are composed largely of cellular elements with an absence of the inanimate intercellular material characteristic of cartilage and bone Muscle and fatty tissue both have a connective tissue stroma which serves as a supporting structure for the muscle and fat cells The fibroblasts in this connective tissue stroma may survive autotransplantation when the muscle and fat cells fail to survive

bone which have regenerative power after injury tend to lose their calcified structure when transplanted in soft tissues but alternately tend to retain their structure when transplanted in contact with living bone

The parenchymal cells in *fat* and *muscle* are the fat cell and muscle cell. The hardy fat cells which succeed in surviving after free autogenous transplantation tend to retain their small amount of amorphous intercellular substance If the cells

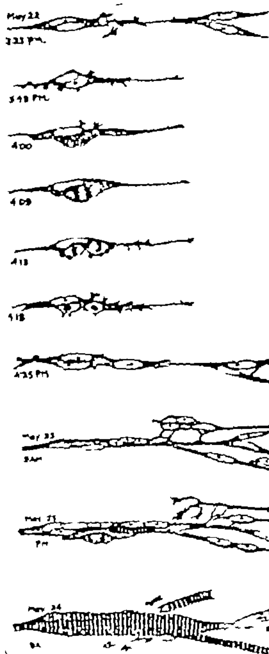


Fig 17 Differentiation of myoblasts into cross striated muscle fibers as seen in living regenerating zone following removal of the tip of a tadpole's tail One of a pair of closely associated myoblasts was watched throughout its nuclear division The next day many nuclei were present At 4 P M the first faint cross striations were visible The following day many cross striations were in evidence in all fibers (Redrawn after Speidel ) (From A A Maximow and W Bloom A Textbook of Histology ed 6 p 160 W B Saunders Company Philadelphia 1952 )

in a fat graft fail to survive the graft will be replaced by fibrous tissue (31)

Muscle cells always degenerate and disappear after free transplantation and the graft is replaced by fibrous tissue. Fat and muscle have a connective tissue support of collagenous and elastic fibers, which extend between muscle cells and fat cells. This fibrous tissue has its own living fibroblast cells, and probably the dead amorphous intercellular substance surrounding the parenchymal cells also pervades and surrounds this fibrous tissue since there is no recognized structure separating the two

The epidermis of the *skin* is similar to fat and muscle in that it contains very little intercellular material. Unlike fat and muscle it does not have a connective tissue stroma supporting its cells and cell groups The parenchymal cell is the epidermal cell, and it tends to survive autogenous transplantation and retain its small amount of intercellular substance. When the epidermal cells fail to survive the graft, being on the body surface sloughs away

The connective tissue dermis with its fibroblast cell serves as a support for the glands and hairs and as a foundation for the epidermis The dermis contains hair follicles glands the

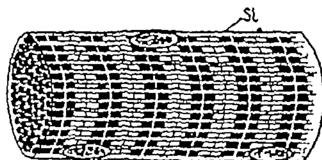



Fig 18 A schematic diagram illustrating the essential structure of a striated muscle fiber as it has been interpreted on the basis of *fixed* preparations According to this interpretation each fiber is composed of a bundle of myofibrillae enclosed in a membranous sheath the sarcolemma (*Sl*) The nuclei of the fiber are situated peripherally immediately beneath the sarcolemma (From W E Le Gros Clark The Tissues of the Body ed 2 p 110 The Clarendon Press Oxford 1945 )



Fig 19 Plain muscle fibers Note their fusiform shape and the elongated central nuclei (From W E Le Gros Clark The Tissues of the Body ed 2 p 139 The Clarendon Press Oxford 1945 )

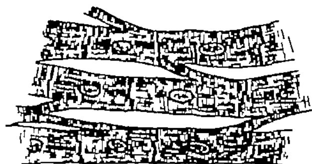


Fig 20 Diagram illustrating the essential features of cardiac muscle Note that the fibers have each a single centrally situated nucleus and that they are in syncytial continuity by side branches The appearance of the intercalated discs is also indicated (From W E Le Gros Clark The Tissues of the Body ed 2 p 144 The Clarendon Press Oxford 1945 )

specialized nerve endings, and pigment forming cells, all of which have parenchymal cells. The fibroblast, with its collagenous and elastic fibers represents the supporting connective tissue stroma for these parenchymal structures In general the glands hairs and end-organs of sensation tend to survive in free autogenous skin grafts when the parenchymal cells in the structures survive.

The pigment forming cell, the melanoblast, also tends to survive and may take on increased melanin production, causing some free skin grafts to become so dark that they form a startling and unpleasant contrast with the surrounding skin.

*Nerves* resemble skin because they contain cell

Fig 21 Diagrammatic drawing of skin with structures in dermis For melanoblasts see Fig 22 A Artery B Vein C Hair follicle D Sebaceous gland E Sweat gland F Ruffini cylinders (receptors of warmth) G Pacini corpuscles (receptors of deep pressure) H Merkel discs (receptors of pain) I Meissner corpuscles (tactile receptors) (Krause end bulbs are assumed to be receptors of cold sensitivity and are usually indistinguishable from Meissner corpuscles ) J Grandry corpuscles K Subcutaneous fat L Axle cylinders (receptors of pain)

and intercellular material of both ectodermal and mesodermal origin. The surgeon transplanting a free nerve graft is concerned with the growth of the axons through the graft and on out to motor endings in muscle or to sense organs in the skin. These axons are cytoplasmic extensions or tails from nerve cells located in some remote ganglion or in the spinal cord The severed nerve axons in a free nerve graft are destined to die in all instances because they have been disconnected from the cytoplasm of their nerve cells

Being necessary for the normal functioning of a nerve axon, the Schwann cells in the neurilemma must be considered as parenchymal cells in peripheral nerve grafts. The myelin sheath, which is a sort of non-living insulator for the axon, is believed to be produced by the Schwann cell The individual nerve axon with its insulating myelin sheath enveloped by the Schwann cell




FIG 22 Section through skin of human mammary papilla *Sc* Stratum corneum. *Pb* pigmented basal cells of epidermis *Mel* melanoblast *Fib* fibroblast *Dc* dermal chromatophore Silver nitrate faintly counterstained with pyronin methyl green ×650 (W B ) (From A A Maximow and W Bloom A Textbook of Histology ed 6 p 316 W B Saunders Company Philadelphia 1952 )

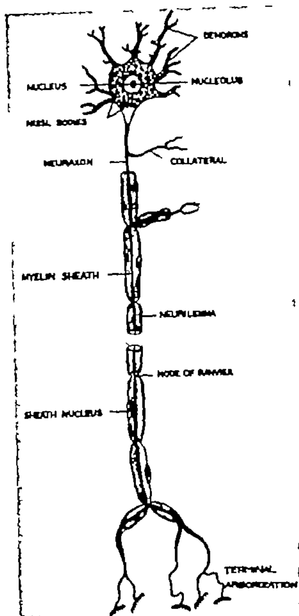



FIG 23 Diagram of a motor nerve cell and investing membranes (From R O Greep Histology p 100 The Blakiston Company New York 1954 )

is supported by a connective tissue stroma This stroma, composed of collagenous and elastic fibers, has its own living fibroblast cell and is designated according to its location as endoneurium, perineurium or epineurium. Proliferation of the fibroblasts in the endoneurium of free nerve grafts often blocks the pathways through which new axons attempt to grow (32)

## Survival of Blood Vessels in Free Autogenous Grafts

The blood vessels with their lining endothelial cells tend to survive in successfully transplanted autogenous human tissue grafts. In fact the

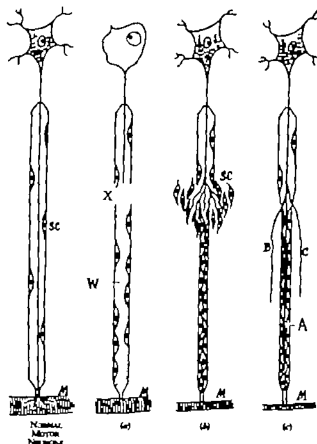



Fig 24 Diagram of regeneration of a peripheral motor nerve fiber SC Schwann cells M muscle fiber myelin sheath left blank and nodes of Ranvier omitted (a ) Fiber recently cut at X Wallerian degeneration of myelin W (stippled) accompanied by some proliferation of Schwann cells swelling and degenerative changes in nerve cell (b ) Recovery of nerve cell multiple regeneration sprouts from proximal cut end of axon proliferation of Schwann cells at gap and in shrunken peripheral neurilemmal tube progressive atrophy of muscle fiber (c ) Regeneration of axon A nearly complete presently it will reach and re innervate muscle fiber unsuccessful collateral branches B and C will then atrophy and disappear (Note the successful fiber A does not necessarily come from the nerve cell which originally supplied this neurilemmal tube it may have come from another and perhaps functionally inappropriate cell ) (From R A Willis The Principles of Pathology p 32 The C V Mosby Company St Louis 1950 )

survival of the blood vessels and the establishment of early circulation must occur (in about three to four days) or the cells in the graft will die from accumulation of waste products, lack of oxygen, and other necessary substances.

This statement, of course applies only to tissues which normally have a blood vessel system of circulation. Cornea cartilage lens, and the epidermis of skin do not have a vascular system and they retain their normal mechanism of exchange by fluid permeation after successful free transfer In free skin grafts after circulation has been established dilated capillaries extend up to the basement membrane but they never penetrate the epidermis, which retains its primitive method of circulation by tissue fluid infiltration.

The blood vessels in the dermis of skin grafts and in buried grafts such as fat (31) tendon (32) bone (33) and other vascular tissues are at first filled with granular debris or in some grafts (fat) with coagulated blood. Very frequently the smaller vessels appear empty in grafts buried for one and two days. During this interval, however the endothelial cells lining the blood vessels appear viable in the stained sections. Usually by the third day circulation has been established in the smaller vessels which are not occluded by coagulated blood, through anasto-

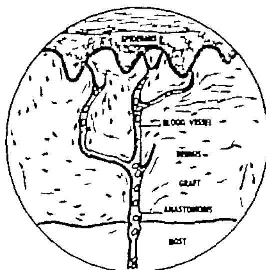



Fig 25 Free skin grafts obtain a blood circulation about 3 days following transplantation through an anastomosis between host and graft blood vessels Small capillary sprouts extend to the basement membrane of the transplant but never appear actually to penetrate the epidermis

At about 5 days there occurs a penetrating ingrowth of capillaries from host blood vessels Possibly these penetrating host capillaries later anastomose with surviving blood vessels of the graft Thus the final circulatory system in the graft may be composed of surviving blood vessels in the transplant supplemented by vessels from the host

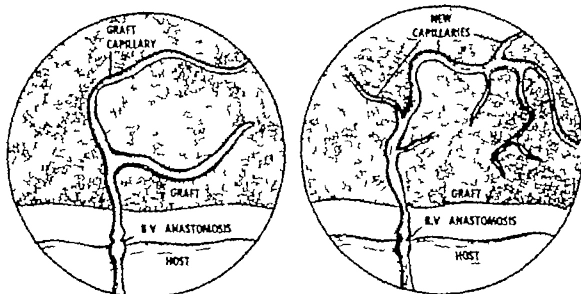



FIG 26 Changing pattern of graft blood vessels in free autogenous skin grafts *Left* Anastomosis has occurred between host and graft blood vessels 3 to 4 days following transplantation *Right* The pattern of distribution of the surviving blood vessels in the graft undergoes change Some graft blood vessels become smaller or disappear while others become larger and send out numerous sprouts to different areas of the dermis Thus the eventual blood vessel pattern in the transplant may be quite different from the original distribution.

mosis between the host and graft blood vessels, although the exact interval varies in different grafts

The anastomosis between graft and host blood vessels is not necessarily an end to-end anastomosis * although this appears to be the pattern of the earliest circulation (established in three to four days) A later penetrating host capillary ingrowth does occur and these host capillaries probably anastomose with small vessels in the graft. Some of the blood vessels in various free grafts are completely occluded by coagulated blood. Many of these occluded graft blood vessels become recanalized and circulating blood is established in seven to eight days after transplantation other occluded graft blood vessels fail to become recanalized and are replaced by connective tissue †

The pattern of distribution of the surviving blood vessels in free grafts, however is not fixed or stable Some unoccluded graft vessels with circulating blood become smaller or disappear small graft capillaries may become much larger and new vessels may appear in response, probably to the chemical requirements of the cells and intercellular substances in the transplanted tissue. *Thus the eventual blood vessel pattern in the graft may be quite different from the original distribution.* ‡

Additional evidence that anastomosis between host and graft blood vessels does occur has been produced by transplanting full thickness skin grafts containing capillary angiomas within the dermis of the grafts. The red color in the skin disappears when the grafts are detached and applied to recipient sites as free autografts. In about three days the capillary angioma reappears indicating that host blood vessels have anastomosed with the vascular system of the graft. Such transfers have been accomplished by the author in four patients and the capillary angiomas have remained in all instances (the oldest case having been observed for six years) §

## THE CELL SURVIVAL THEORY

A study of the behavior of four of the five free autogenous tissue grafts described in Volume

* Due to an oversight a contrary statement appeared in volume 1

† See Transplantation of Tissues volume 1 chapter 30

‡ Unpublished observations regarding the circulation in autogenous animal skin grafts by Sven Bellman at the Serafimerlasarettet and Karolinska Institutet Stockholm Sweden These interesting findings were demonstrated to the author by Dr Bellman through the courtesy of Dr Allan Ragnell in August 1955

§ See Transplantation of Tissues volume 1 chapter 30

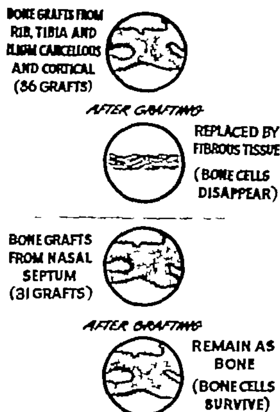



FIG 27 Author's experimental series illustrating the fate of bone grafts in contact with unlike tissues (fat) *Upper* Thirty-six living autogenous human rib tibial and iliac bone grafts were all replaced by fibrous tissue in 6 to 8 months following transplantation *Lower* Thirty-one living autogenous human septal bone grafts all remained as bone with living bone cells up to 5 years following transfer Turbinate and nasal bone autografts also retain their calcified matrix when buried in subcutaneous fat

1 indicated that the cells *show a tenacious* ability to survive as living entities. In grafts of cartilage cancellous bone in contact with bone septal, nasal and turbinate bones, fascia, and tendon, the cells tend to survive and retain the specific structure of their matrix. The muscle cells in free muscle grafts die in all transplants, but the graft structure is replaced as fibrous tissue and not as muscle. There is evidence that many fat cells in free adipose transplants survive, and fat grafts which fail to survive are probably replaced by connective tissue rather than by new fat cells (31) In free nerve grafts the fibroblasts in the connective tissue stroma both *survive* and *proliferate* and positive evidence that the Schwann cells do not also survive is lacking (32) The epidermal cells in free skin grafts are known to survive and few investigators question the survival of fibroblasts in the dermis. Specialized end-organs of sensation the hair follicles and glands, and the melanoblasts tend to survive largely in free skin grafts. When skin transplants fail to survive, these specialized structures are not replaced by host tissues with the possible exception of melanoblasts.

The generally successful effort of the cells in most free autogenous human grafts to survive as living entities is in broad perspective an impressive fact. A postulated cell survival theory' may apply to all of the human tissues considered in Volume 1 with the exception of dense cortical bone. It may also apply to other commonly used tissue grafts described in the present volume such as fat, skin, and possibly peripheral nerves.

The cell survival theory is stated as follows *In humans the cells in free autogenous grafts tend to survive and retain their normal tissue structure when transplanted as complete cell entities in favorable transplantation sites When the cells in free grafts fail to survive the graft is replaced by connective tissue but this replacement is not a duplicate of the original graft.*

## BEHAVIOR OF CELLS IN TISSUE TRANSPLANTS

### Cell Behavior in Autografts

It is not possible to make any statement about the behavior of the cell population in autogenous transplants without limiting qualifications regarding individual grafts and regarding changes which occur over periods of time.

*For example the normal life span of the fibroblasts* in the dermis of skin grafts as well as that of the fibroblasts in tendon and fascia is not known. Presumably there is a normal turnover in these cells, so that in transplantation of a piece of autogenous skin the fibroblast population consists of young, middle-aged, and old fibroblast cells with different life expectancies, similar to the situation of red blood cells in transfused blood. After successful transplantation of the autogenous skin graft and initial survival of the dermal fibroblasts there may later occur a gradual replacement of these cells either through infiltration of young host fibroblasts or by cell division of young fibroblasts in the transplanted dermis this may approximate the normal cycle in untransplanted skin.

Specialized methods of marking the fibroblasts

in the transplanted dermis and noting that these marked cells gradually disappear have not been correlated with similar studies regarding the behavior of fibroblasts in the dermis of skin *in situ* which has not been transplanted.

Thus the term "cell survival" as applied to the cell population in autografts means that the cells in the transplants survive the initial shock of the procedure and do not suddenly undergo mass death such as occurs in the cells of soft tissue homografts which are not protected by avascular matrix material (as in cornea and cartilage)

The cell survival theory does not exclude the probability that each specific tissue follows its normal physiologic cycle of cell senescence and death and new cell replacement. Possibly the stress of free transplantation hastens this cycle but replacement often occurs by mitosis of cells in the graft rather than from infiltrating host cells. This is clearly demonstrated by the initial survival and later cell division occurring in the epidermis of transplanted skin autografts. The endoneural fibroblasts in free nerve grafts *not only survive but also proliferate to such an extent that they often block the pathways through which axons from the proximal nerve attempt to grow* *

The theory of gradual replacement of free autogenous grafts by infiltrating host cells in such a manner that cartilage is replaced by cartilage, dermis by dermis, fascia by fascia tendon by tendon and even epidermis and its derivatives (hairs and glands) by epidermis, has dominated the thinking of past investigators and still influences the reasoning of present day experimenters and clinicians.

The great activity of the fibroblast in wound healing and the fact that certain bone grafts in contact with host bone can be replaced by new bone formation from the host were noted by many early investigators It was quite natural therefore that these men should believe as a generalization either that free grafts were absorbed and replaced by connective tissue or that, if the graft structure remained host tissue cells had gradually infiltrated the grafts and replaced the original cell population. The replacing cells from this viewpoint possibly elaborated new intercellular substances in such a clever

* See Transplantation of Tissues volume 1 page 328

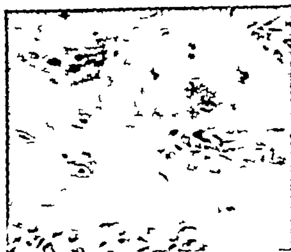


FIG 28 Autogenous human nasal bone graft buried in abdominal wall fat for 5 years Note that calcified matrix is present and that bone cell appear viable in this fixed and stained section



FIG 29 Autogenous human septal bone buried in abdominal wall fat for 4½ years calcified matrix which has become quite d and sparsely scattered bone cells

way that the "counterfeit model" was an duplicate of the original graft.

The cell responsible for the replacem many free grafts of mesenchymal org believed by some to be the ubiquitous un entiated mesenchymal cell. These cells, acc to histologists, are often smaller than fibr but have the same general appearance loose connective tissue they are usually ar along the blood vessels, particularly amc capillaries. Cowdry (34) very aptly remar the carry-over primitive mesenchymato are *so convenient to think of and so hard* Certainly great vagueness is manifest i

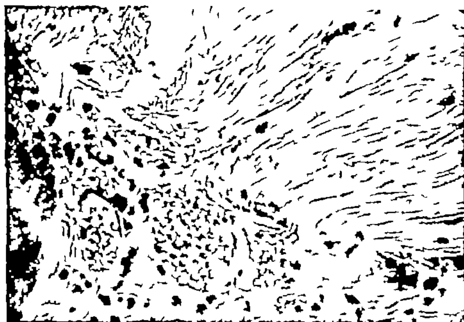


FIG 30 Autogenous human tendon graft buried in abdominal wall fat for 3 days Note engorged graft blood vessels demonstrating that anastomosis has occurred with host vessels 3 days following transplantation The tendon cells appear normal but there is an extravasation of host white blood cells into the graft in the area around the engorged blood vessels

ments concerning what tissues the mesenchymatous cells accurately replace and why they replace the viable cells in free grafts which have survived the initial shock of transplantation In effect positive evidence supporting host replacement of the cells in many free tissue grafts that have survived the stress of the transplantation procedure is obscure.

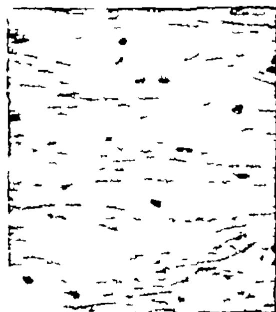


FIG 31 Autogenous human tendon graft buried in abdominal fat for 10 days There are occasional polymorphonuclear leukocytes scattered throughout the graft but the tendon cells appear normal with no evidence of dead or degenerating tendon cells Autogenous human tendon grafts buried for longer periods of time also fail to show degeneration and death of the graft cell

## Cell Behavior in Homografts

Although this subject is thoroughly presented by Prof Medawar Dr Rogers, and other contributors it seems pertinent to describe here some of the more important behavior patterns of homografts which are of practical interest to clinicians. Our knowledge of homografts and the various theories advanced to explain the 'rejection phenomenon' are undergoing constant change and there is considerable difference of opinion among research workers as to all of the factors involved.

The generally accepted theory regarding the rejection of homografts is that of an actively acquired immunity According to this concept the homograft liberates some product which stimulates the formation of a specific antibody This antibody causes destruction of the cells in the transplant and gradual necrosis of the graft (in soft tissue transplants such as skin)

The cells in cartilage and lens homograft and in the opinion of some fibrocytes in the interstitial portion of nonvascularized corneal homografts survive for much longer periods of time because the graft cells are protected from



FIG 32 Heterogenous cartilage graft (sting ray) transplanted in human abdominal wall fat for 8 months Note dense cellular infiltration which has divided the graft into separate pieces to facilitate absorption

Cartilage autografts tend to be tolerated with viable cells cartilage homografts are in general slowly absorbed and cartilage heterografts are more rapidly penetrated and removed by the host tissues The gelatinous matrix of cartilage like the interstitial layer of cornea is quite durable and it may persist for long periods of time in homografts The chondrocytes in cartilage homografts appear to remain viable as long as they are protected by their matrix

hostile host cells by their gel-like matrix and because these tissues are avascular

Any theory of an actively-acquired immunity would be obliged to demonstrate that a second crop of homotransplants from the same donor to the same recipient would disintegrate at a a faster rate than the first Medawar (35) demonstrated exactly this a second crop of skin homotransplants from the same donor to the same recipient did disintegrate at a faster rate than those of the first crop This is strong evidence favoring the antigen-antibody theory in the rejection of skin homografts.

A circulating serum antibody which will destroy homogenous cells however has not been demonstrated according to most investigators. Host cells, apparently must be present in homografts to effect destruction of the transplanted cells possibly these host cells which have been identified as lymphocytes carry the antibody The fact that host cells have difficulty in rapidly penetrating the matrix of homogenous cartilage may explain the long survival time of the chondrocytes in these homotransplants *

* Transplantation of Tissues volume 1 chapter 8

Harry S. Greene (36) of Yale is not convinced that the rejection of homografts is based on an antigen-antibody phenomenon similar to bacterial antigen-antibody formation He suggests that the terms compatibility and incompatibility in transplantation require qualifications in relation to factors pertaining to the transplant and the transplantation site as well as to the constitution of the donor and recipient. The general statement that the tissues of a certain species strain or individual are incompatible with those of another disregards both the status of the tissue and the site of transplantation and derives largely from considerations based on *adult tissues* as solely representative of the donor and on *the subcutaneous space* as solely representative of the recipient. Actually Greene continues the status of a tissue varies with development and may be embryonic or cancerous as well as adult Further the body of the recipient extending beneath the subcutaneous space contains areas of differentiation such as the eye, and brain whose special attributes provide the tissue with more suitable conditions for growth but do not confer an independence of the constitutional factors determining the reaction to transplantation

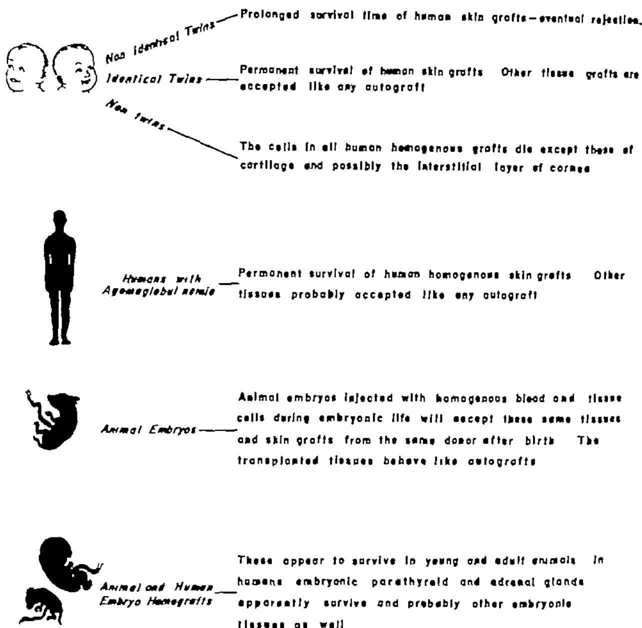



FIG 33 Behavior of homografts The prolonged survival of skin exchanged between non identical twins has been reported as 20 days This however merely approximates the tolerance of some skin grafts exchanged between father and infant and does not meet the author s standard of relative tolerance which is a minimum of 30 days

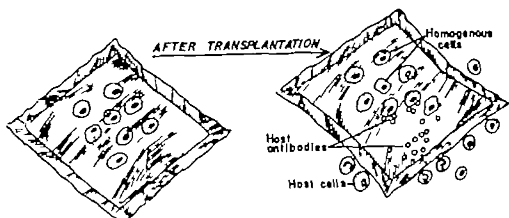
The eye and brain are parts of the body and are not unconstitutional in response to transplantation.

The adult *state of a tissue represents*, in Greene s opinion, a development phase and not its life history The initial state of all tissues is embryonic and a terminal state may be cancerous. Compatibility relationships vary accordingly

Homogenous skin grafts have been studied extensively in animals and in human beings and their behavior pattern has been found to approximate that of other homografts which normally have a blood vessel circulatory system.

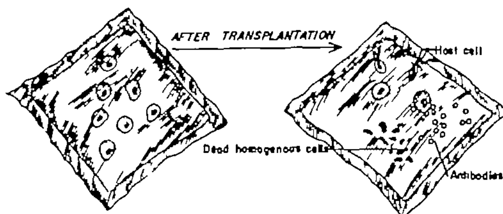
Homogenous skin grafts in animals develop a blood circulation by the third or fourth day According to Taylor and Lehrfeld (29) a *stasis of blood occurs in the graft* blood vessels in rat skin homografts between the seventh and ninth day following transplantation. This in their opinion, marks the end point of survival or beginning of rejection of the skin homograft.

In human beings the skin homotransplant also develops a circulation by the third or fourth day but the rejection of the graft *does not follow such a definite time schedule* Although many human skin homografts show *gross evidence of*




Homogenous cells in diffusion chamber with small pores which admit antibodies but will not admit host cells

The homogenous cells survive Host antibodies flow through small pores and probably form connection with cells but these remain viable because the host cells are absent




Homogenous cells in diffusion chamber with large pores which admit both host cells and antibodies

Host cells in combination with host antibodies destroy the homogenous cells

FIG 34 Diagrams illustrate the behavior of transplanted homogenous and heterogenous cells which are protected from hostile host cells Homogenous cells protected from host cells survive like the chondrocytes in cartilage homografts and possibly the fibroblasts in corneal homografts both of which are protected from host cells by their avascular matrix material (J M Weaver G H Algire and R T Prehn The Growth of Cells *in Vivo* in Diffusion Chambers J of Nat Cancer Inst 15, no 6 1955)

beginning rejection between the seventh and ninth day others persist for much longer periods of time For example the author and his associates have exchanged full-thickness skin grafts between parents and infants who belong to compatible blood groups in regard to the ABO Rh factor *All skin exchanges between fathers and boy or girl infants were rejected by both recipients* but some grafts persisted grossly for 20 days When the exchange of skin was made between mothers and infants *there were many long-surviving homografts* on the basis of gross examination (up to 28 months)

Three long-surviving skin homografts were observed not only with mother's skin on infants but also surprisingly in cases of *infants skin on mothers* Two biopsy sections removed at 20 days and 8 months respectively following transplantation demonstrated normal *epidermis* and unoccluded blood vessels with apparently normal

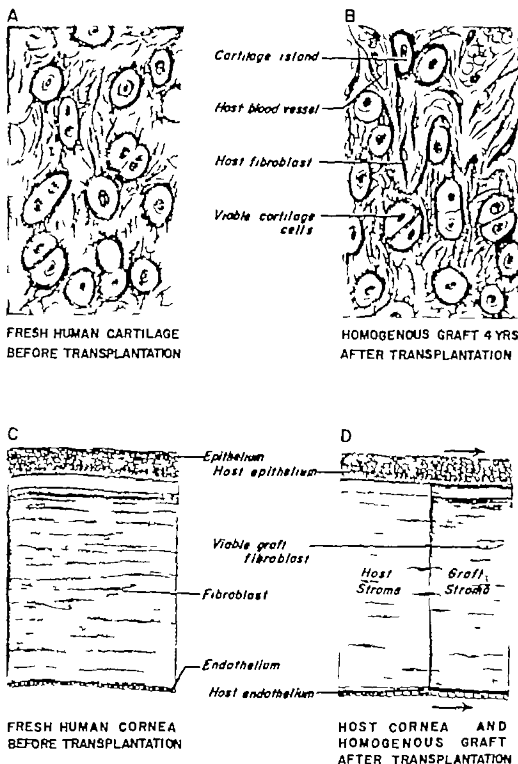



*Fig. 35. The drawings A and B demonstrate the behavior of a fresh human cartilage homograft 4* years after transplantation. The cartilage cells remain viable as long as they are protected by even a thin armor of matrix which excludes hostile host cells. When all of the matrix is absorbed the exposed cartilage cells are destroyed.

The drawings C and D demonstrate the behavior of fresh corneal homografts which are not invaded by blood vessels according to some authorities. The epidermis and endothelium are replaced but the fibroblast cells in the thick interstitial layer survive as living cells if the graft remains transparent. If the corneal graft becomes opaque because of the ingrowth of host blood vessels the fibroblast cells are replaced by host cells. Other authorities believe that the fibroblasts in the interstitial layer of the cornea are always replaced by infiltrating host cells.

It would seem that sex chromatin studies on sections of cartilage and corneal homografts might determine the survival or replacement of the transplanted cells when the grafts are made between males and females.

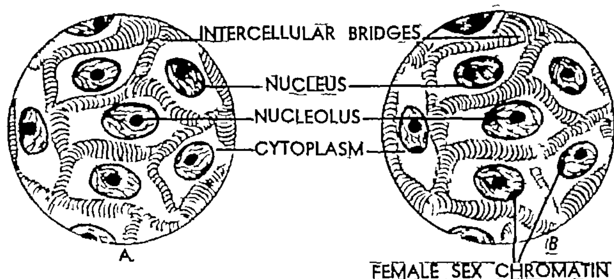



FIG 36 A Male epidermal cells which do not contain the female sex chromatin B Female epidermal cells showing characteristic female sex chromatin body in contact with nuclear membrane

Sex chromatin study affords a positive method for determining the survival of homogenous skin grafts when the donor and recipient are members of opposite sexes The method may also be useful to determine the survival of cells in cartilage and corneal homografts

endothelial cells and red blood cells Hairs and glands were present in the 20-day homograft but were absent in the homograft transplanted for 8 months. This older graft also had *a dense infiltration of lymphocytes within the dermis* The epidermal cells in both of these biopsy sections showed the sex chromatin which is characteristic of female skin (37) Since the transfer was from mother to boy infant, the skin was the mother s rather than replacement skin from the male infant. The author has applied Murray Barr s chromosomal sex determination in all cases of tolerated skin grafts between male and female donor recipients and believes that this is strong evidence in determining the survival or host replacement of skin homografts (30 38)

In two instances exchanges of skin were made between mothers and their 7 and 12 year-old male children. These homotransplants have been retained by both pairs for 78 and 253 days respectively which indicates that tolerance may persist as children become older Biopsy studies made of the homograft from mother to 12 year old boy three months after transplantation demonstrated the sex chromatin in the epidermal cells which is characteristic of female skin Biopsy study of the boy s graft on the mother showed an absence of female sex chromatin These transplants however *did not appear like autografts* The epidermal layer was normal but there was an absence of hairs and glands and the dermis was infiltrated by a dense collection of lymphocytes

The instances of longer survival of skin homografts from mother to child compared with those of father to child suggest that tolerance between mother and child may occur because of intermingling of fetal and maternal circulations during the mother s pregnancy The early rejection of grafts interchanged between mother and child in other instances may be explained by a

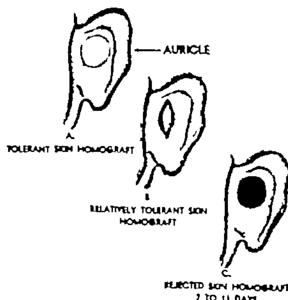



FIG 37 *Skin grafts exchanged* between mothers and children may demonstrate early rejection as an acute phenomenon or may be relatively tolerant showing considerable contraction pigmentation and superficial bleb formation Occasional homo-skin grafts appear grossly to be tolerated like autografts for long period of time



FIG 38 Mother boy infant skin graft exchange 213 days after transplantation. The infant did not receive a blood injection and was grafted when 2 months old *Left* Mother's skin behind infant's ear shows relative tolerance having undergone considerable contraction pigmentation and thinning Superficial blebs appeared in the center of this graft 40 days after transplantation but these later completely healed *Right* Child's skin behind mother's ear has been well tolerated

more complete separation of maternal and fetal circulations during the pregnancies of these mothers. Long survival of the infant's skin on its mother is difficult to explain.

Intramuscular injections of 16 infants from 3 to 28 days old with a parent's blood did not appear to render the child more tolerant to skin from the donor parent long-surviving homografts were observed also in an uninjected control series of 33 skin exchanges but only when the exchange of skin was made *between mothers and infants* (30) Woodruff (39) however observed relative tolerance to father's skin in two infants who had been



FIG 39 Mother boy-infant skin graft exchange 209 days following transplantation The infant received an injection of its mother's blood when 4 days old and the skin was exchanged 42 days later *Left* Mother's skin behind infant's ear has been well tolerated *Right* Child's skin behind mother's ear also well tolerated with little scarring contraction or color change

injected intramuscularly with a large amount of antigen (concentrated leukocytes) very shortly after birth (3 hours and 48 hours)

Injection procedures have been followed on the assumption that pretreatment with cells from a prospective skin donor might create a state of tolerance in young infants similar to that observed in the experimental work of Medawar Billingham, and Sparrow (40) These authors injected fetal mice and chicks with living homogenous cells and thereby created tolerance to skin grafts from donors that had provided the inoculum. Woodruff (41) later demonstrated that tolerance could also be conferred on newborn rats by pretreatment with cells from prospective donor rats. There may however be some danger associated with the injection of large numbers of living homogenous cells into newborn infants and the author agrees with Woodruff that this matter should be clarified by further animal experimentation

Children who are tolerant of their mother's skin may also tolerate other tissues from the mother *such as kidney and endocrine* glands. Routine skin exchanges between mothers and children, therefore, may prove to be useful tests for possible tolerance to other tissues exchanged between mother and child.

It has been observed that human beings with severe burns appear to tolerate homogenous skin grafts for much longer periods of time than patients without burns who are grafted experimentally This has given rise to the clinical assumption that homogenous skin grafts are especially well tolerated by patients who have a physiologic need for skin eventually however the homogenous skin grafts are rejected by these patients

Good and Varco (42) observed that patients with agammaglobulinemia tolerate homografts of skin and other tissues like autografts. This important finding is further evidence that the rejection phenomenon of homografts is on the basis of an antigen-antibody formation.

Tolerance of homoplastic grafts may be *relative* or *complete* In relative tolerance a skin graft will show a reaction that is too weak to destroy the grafted cells. This reaction is indicated by a tendency to build up fibrous tissue and to undergo contraction and an eczematous deterioration of the epithelial surface. Such partially tolerated grafts may recover from the crisis and persist for long periods of time, or they may gradually go

on to further deterioration and eventual complete replacement by host tissues. The late rejection of partially tolerated homografts, therefore is not an acute rejection phenomenon such as occurs in homografts which are destroyed between the eighth and fourteenth day following transplantation. Homografts which are fully tolerant behave like autografts.

The longer a homograft is tolerated, the more likely it is to survive for some further period of time. In the author's experiments the human skin homografts were considered as revealing relative tolerance when they survived for 30 days. The oldest graft in the series has been well tolerated for 28 months and appears grossly like an autograft, showing no appreciable contraction and no discoloration or surface excoriation. This homotransplant was from injected boy infant to mother. This same infant tolerated a skin graft from his mother for 3 months and then gradually rejected the skin (relative tolerance).

As pointed out by Billingham, the survival time and cosmetic properties of a skin graft can be relied upon to show up the whole spectrum of compatibility, not excluding the weakest immunity or (which comes to the same thing) a tolerance which is almost but not quite complete.

### Sex Chromatin to Determine Cell Survival in Homografts

A sex difference in nuclear structure was first described by Barr and Bertram (37) in 1949. Nuclei of the hypoglossal neuron cell of female cats was shown to contain a chromatin mass, about one micron in diameter which was seldom seen in male cats. Moore and Barr (43) in 1953 found a sex difference in nuclear morphology similar to that described earlier for the cat, in nerve cells of the dog, mink, martin, ferret, raccoon, skunk, goat and deer. A sex difference could not be seen in nerve cells of certain rodents such as guinea pig, rat, mouse, hamster, ground hog, and rabbit.

Later work demonstrated that the nuclei of epidermis and many other tissues of the female cat contain sex chromatin at the nuclear membrane. The epidermis of human female skin also has the sex chromatin marking and from this observation there developed a test of chromosomal sex in hermaphrodites (44). The average incidence of sex chromatin in sections of human epidermis stained with H and E is 60 per cent in females and 5 per cent in males.



FIG 40 Mother–boy infant skin graft exchange 321 days after transplantation. The infant received an injection of its mother's blood when 4 days old and skin was exchanged 42 days later. Both grafts took initially but the mother's skin on the child was gradually completely rejected and replaced 90 days after transplantation. Photograph shows child's skin behind mother's ear which appears well tolerated 321 days following transplantation.

Further experiments in this provocative study (45) demonstrated that the nuclei of various human female tissues have a fairly conspicuous mass of chromatin, the sex chromatin, which lies against the inner surface of the nuclear membrane in most instances. The nuclei of human female cartilage cells also have the characteristic sex chromatin marking.

In both epidermis and cartilage the sex chromatin usually located next to the nuclear membrane has a planoconvex outline. In other nuclei the sex chromatin is disc-shaped or triangular and occasionally has a circular or bell-shaped outline. In some instances however the sex chromatin may be visualized as a free body not in contact with the nuclear membrane, hence the term "nucleolar satellite" has been applied to this particle.

Such evidence as is available indicates that the sex chromatin of female cells may represent a *fusion of portions of the two X chromosomes* while the XY sex chromosome complex of male cells fails to form a chromatin mass of comparable size.

### *Skin Homografts*

Survival of the epidermis of tolerated skin grafts exchanged between mothers and male

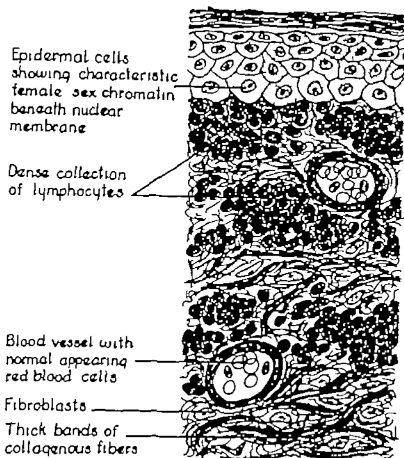



*Fig 41* **Drawing of high power magnification of homogenous skin graft transplanted from mother to boy-child Survival of the mother's epidermal cells can be determined by the characteristic sex chromatin The hairs and glands have disappeared and the dermis is infiltrated by a dense collection of lymphocytes This graft was removed for biopsy study 8 months following transplantation**

(Drawn by Miss Beth Sherman)

children can be determined by the presence or absence of the female sex chromatin. The author's group has made biopsy studies of these homotransplants 20 78, 240 and 253 days following grafting and observed the sex chromatin in female epidermis and its absence in male epidermis. The hairs in these tolerated homografts tended to degenerate slowly and glands were absent in skin grafts transplanted for 78 days and longer The dermis was infiltrated by host lymphocytes.

### *Cartilage Homografts*

*The persistence* of the *chondrocytes in fresh* homogenous cartilage grafts (40) can be clearly demonstrated by the sex chromatin markings when *transplants are exchanged between male* and female patients. Septal and alar cartilage serve particularly well for this experimental purpose, *because they are relatively cellular and* quite thin which favors satisfactory fixation and staining. The plump fibroblast like cells near the periphery *of female homotransplants will have* the sex chromatin particle in about 50 per cent of the cells. Sex chromatin will be absent or only occasionally visualized in male cartilage transplants.

### *Corneal Homografts*

The author and his associates have demonstrated the presence of the sex chromatin body in the nuclei of the epidermal layer of cornea in human beings and in cats and its absence in the cornea of rabbits. This confirms Moore and Barr's observation that sex chromatin is present in human and cat skin but absent in the skin of many rodents. It would therefore seem possible to determine rather accurately the survival or replacement of the epidermal layer in homogenous corneal grafts by exchanging transplants between male and female cats.

### Clinical Application

What then are the facts of clinical value regarding *homografts that the surgeon may safely*




INJECTED INFANTS — 11 SKIN EXCHANGES
SURVIVAL — SKIN ON 2 MOTHER AND 2 INFANT PAIRS
SKIN ON 2 INFANTS
SKIN ON 2 MOTHERS
REJECTION — SKIN ON 5 MOTHER AND 5 INFANT PAIRS
UNINJECTED INFANTS — 17 SKIN EXCHANGES
SURVIVAL — SKIN ON 1 MOTHER AND 1 INFANT PAIR
SKIN ON 2 MOTHER AND 2 CHILD PAIRS
SKIN ON 3 INFANTS
SKIN ON 2 MOTHERS
SKIN ON 1 CHILD
REJECTION — SKIN ON 6 MOTHER AND 6 INFANT PAIRS
SKIN ON 2 MOTHER AND 2 CHILD PAIRS
NO APPARENT RELATIONSHIP REGARDING BLOOD GROUPS, A. B. O. Rh. and GAMMA GLOBULIN LEVELS.




INJECTED INFANTS — 2 SKIN EXCHANGES
SURVIVAL — SKIN ON 1 INFANT
REJECTION SKIN ON 1 MOTHER AND 1 INFANT PAIR
UNINJECTED — 10 SKIN EXCHANGES
SURVIVAL — SKIN ON 3 INFANTS
SKIN ON 1 MOTHER
— SKIN ON 1 MOTHER AND 1 INFANT PAIR
REJECTION — 5 MOTHER AND 5 INFANT PAIRS
NO APPARENT RELATIONSHIP REGARDING BLOOD GROUPS, A. B. O. Rh. and GAMMA GLOBULIN LEVELS.




12 SKIN EXCHANGES.
INJECTED AND UNINJECTED INFANTS —
SURVIVAL — NONE BY INFANTS AND FATHERS REGARDLESS OF BLOOD INJECTION. LONGEST SURVIVAL 20 DAYS.
NO APPARENT RELATIONSHIP REGARDING BLOOD GROUPS A. B. O. Rh. and GAMMA GLOBULIN LEVELS.

Fig 42

utilize as accepted advances in medical and surgical care? These may be listed as follows

1) Homografts may be exchanged between identical twins with the expectation that the transplants will behave like autografts of similar tissues.

2) *Homografts* transplanted to patients with agammaglobulinemia are tolerated like autografts.

3) In severely burned children it may be expedient to use the mother's skin rather than skin from the father or from other donors. In the ex

perimental series of the author's group about one-fourth of the infants tolerated skin grafts from their mothers for 30 days or longer when blood groups were compatible. *All exchanges of skin between fathers and infants were rejected*

This tolerance of some children for their mother's skin appears to remain as the child grows older (up to 12 years of age in the author's experience) and is not dependent on a low gamma globulin level.

4) Homogenous grafts of cornea may be used with clinical success.

5) Preserved or fresh homogenous arterial grafts are successfully used by those engaged in blood vessel surgery Some investigators however are attempting to utilize preformed autogenous tubes for the replacement of arterial vessel segments in the belief that these are preferable in young children with long life expectancy

6) Fresh or preserved cartilage homografts have a definite place in surgery but cartilage autografts are preferable. The same statement may be made regarding fresh and preserved cancellous bone grafts.

7) Blood transfusion is the most commonly used homotransplantation The living white blood cells in the transfused blood are destroyed in a matter of hours possibly by the liver The red corpuscles, which are considered non-living bags of oxygen carrying hemoglobin, continue to function in this capacity for about 90 days.

8) Unerupted secondary tooth buds transplanted to *adult human beings will continue their* growth cycle and develop into a small adult tooth structure. The tooth buds are supernumerary in the donors and therefore can be removed and transplanted into bony openings formed in the jaws of individuals who have had a tooth extracted. The tooth buds retain their specificity after transplantation, so that canine buds develop into canine teeth molars into molars *et cetera.*

Transplanted homogenous tooth buds become firmly set in the alveolar bone and their development is favorably influenced by functional use such as chewing. Transplantation of homogenous tooth buds is an old procedure which has been discarded, because authorities believe that eventually (after one to three years) the transplanted teeth are rejected. The longest survival (or functioning) time of a homogenous tooth bud in the experience of the author's group* is two years in a female recipient, and this tooth still firmly set in the alveolus causes no discomfort to the patient.

Children with cleft palate often have supernumerary teeth. If test skin grafts transplanted from child to mother have a long survival time in these cases, it is possible that tooth buds might also be tolerated for periods of time longer than reported survival in randomly selected donor and recipient.

9) The grafting of homogenous organs, endocrine glands peripheral nerves and embryo tissues is still in the field of experimental surgery The behavior of these transplants is described in other chapters of the present volume

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* Dr Miklos Csernfalvi Saint Barnabas Rehabilitation Center Dr Fleming in the present volume gives a complete and critical review of animal experimental work with transplantation of teeth An unerupted tooth bud is an embryonal carry-over into postfetal life with considerable growth potential Biologists have studied the behavior of cartilage and corneal homografts extensively but they seem to have neglected the homogenous tooth bud

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CHAPTER 2

# Zoologic Laws of Transplantation

P B MEDAWAR

## INTRODUCTION

Transplantation as the surgeon understands it is an act that has no counterpart in nature. There can therefore be no 'zoologic laws of transplantation' in the sense in which there are laws of heredity or of development and growth. The laws of heredity purport to provide a causal theory of what actually happens when plants and animals perpetuate their kind. The laws of transplantation have no such natural foundations to rest upon; they consist of a number of more or less trustworthy empirical generalizations about the consequences of performing a certain kind of experimental act. Even the fact that free transplantation is possible is an experimental finding, not a matter of mere observation. Nor is it legitimate to argue that transplantation, although indeed a thoroughly artificial process, is one which promotes or hastens or reinforces natural repair. On the contrary, transplantation is used in surgical practice when regeneration fails or is altogether wanting. It is not an adjuvant of natural repair but a most ingenious substitute for it. It will be reasoned later, for example, that skin grafting in human beings owes a large part of its success to the fact that it stops the inept process of 'natural' healing altogether and substitutes a better in its place.

To describe the science of transplantation as empirical is to classify the way in which its information has at present come by. It is not a criticism of, nor even a comment upon, the quality of that knowledge itself. But it is probably true to say that in an empirical science such as this the design, conduct and faithful reporting of experiments are more important than the exact meaning which may be read into their results. If the facts are right, a valid theory will sooner or later accommodate them. Osler said, or may well have said, that no facts are so difficult to explain as those which are not the case.

### Kinds of Grafts

Grafts are classified by the taxonomic relationship between their donors and recipients (autoplastic, homoplastic, heteroplastic, xenoplastic); by the anatomic relationship between their origin and destination (isotopic, orthotopic, heterotopic); by the way in which they are carried out (e.g., free, partially attached); by whether or not they persist as living entities (homovital, homostatic, in Longmire's terminology); and in a variety of other ways that need not be described by special terms.

Autoplastic grafts are those of which the donor is also the recipient; grafts are homoplastic when the recipient is some other member of the donor's species. Grafts transplanted between members of different species are described as heteroplastic, but if the taxonomic gap between donor and recipient becomes wide enough it will make room for a special term, xenoplastic. An orthotopic graft is one transplanted to an anatomically proper environment (skin into gap in skin, for example, or nerve into gap in nerve); the word isotopic distinguishes those orthotopic grafts which enjoy an exact topographic correspondence. Grafts in unnatural positions are heterotopic; for example, grafts of thyroid, skin or cartilage beneath the integument or in the brain or the anterior chamber of the eye. Free grafts, unlike pedicled grafts, have at some time been wholly severed from the body. Homovital grafts start alive and, if they are to deserve their classification, remain so; but

homostatic grafts are progressively revitalized by the tissues of their hosts.

Most systems of classification are vulnerable to determined criticism and that which has just been outlined is no exception. Autografts homografts and the rest are best thought of not as qualitatively distinct categories but as adjacent stretches on a virtually continuous spectrum of zoological affinities. Grafts transplanted between identical twins, or between members of very highly inbred strains of guinea pigs or mice, are homografts by the letter of the definition but not the spirit genetically they are autografts or a very close approximation to them. Homograft and heterograft are equally indistinct at their common boundary A taxonomist of the kind called a 'splitter' may convert a homograft into a heterograft by causing what has hitherto been a single species to be divided into two No recognized degree of disparity separates heteroplastic from xenoplastic grafts

It is equally possible to carp at the distinction between homovital and homostatic grafts. Some grafts must be alive on transplantation and must remain so if they are to serve any permanently useful purpose these are homovital. Other grafts (for example frozen-dried bone or arterial segments) need not even be alive to begin with. Between these extremes are grafts which persist in part and are in part replaced (Maumenee's interpretation places the corneal graft within this category) Here too then, it may be best to think of a series of complementary degrees of survival and replacement. But although it may be best to *think* of the classification of grafts in this way one cannot very well speak or write in terminologic gradients. There can surely be no harm in speaking of homostatic or homovital grafts provided that boundary cases are distinguished by suitable qualifications.

### Scope of Chapter

We are here concerned mainly with lower animals. The evidence that lower animals give of men is fallible but it is not *peculiarly* fallible. The indiscretion of arguing from animals to man is only a special form of the indiscretion of arguing from one species to another We shall find plenty of examples of constitutional differences between the members of different species. Rabbits respond to cortisone quite otherwise than guinea pigs. Mice can be immunized to tissue antigens by the intravenous injection of blood leukocytes rabbits apparently cannot. In the inflammatory processes that accompany the breaking down of homografts, guinea pigs resemble men more closely than they resemble rabbits and rabbits are more like cattle than rats. The argument from mice to men differs from the argument from mice to monkeys only in its being charged with a greater responsibility—a big difference, and one which may be of literally vital importance, but of a moral rather than a factual kind.

The greater part of this chapter deals with skin which provides the simplest of all orthotopic grafts and yet the most exacting of all homografts in the conditions which must be fulfilled if the grafted tissue is to survive longer than a matter of weeks. A discussion of the natural history of healing of wounds of the integument will lead to the conclusion that skin grafting is an operation forced upon the plastic surgeon by the peculiar ineptitude of natural repair as it occurs in the skin of man. The argument will then turn to the problem of repairing defects in skin which are too large to be covered in their entirety by skin autografts, and it will be shown that many of the solutions which are possible in practice are unsound in theory while the one solution which is theoretically desirable—the use of homografts—is not yet possible in practice. The theory of transplantation immunity is dealt with at some length and the behavior of skin under homoplastic transplantation is made the baseline of a comparison with the behavior of other tissues. Exact bibliographic citations are confined to the more recent or less familiar literature, for the science of transplantation is now well served by symposia and reviews (1)

## HEALING OF SKIN DEFECTS IN MAMMALS

A full thickness skin defect in a human being, square in outline and ten square inches in area might well be thought suitable for grafting—an implied acknowledgment of the fact that it could not be relied upon to heal satisfactorily of its own accord. The surface area of a large rabbit is only one-sixth or one-seventh that of a human being yet a full thickness defect of just this size will heal admirably without grafting, and will leave nothing to mark its former position except a clean >—< shaped line of union.




FIG 43 Four successive stages in the healing of a wound in the skin of a rabbit by a combination of contraction and intussusceptive (intercalary) growth

A Day of operation Skin has been removed down to the level of the panniculus carnosus leaving a rectangular island still attached centrally

B After 20 days The entire raw surface is now covered by a thick sheet of migratory epithelium arising partly from the wound margin and partly from the central skin island This epithelium will disappear Note that the margins of the wound are beginning to cave in

C After 27 days A further development of the condition illustrated by B Note that the skin edges at the angles of the wound have now begun to come together the central skin island is beginning to expand.

D After 48 days Contraction is complete and the original wound margins have come together obliterating the migratory epithelium The central skin island fully haired has expanded to about five times its original area (Millimeter scale attached.)

There are two ways of looking at this difference. On the one hand, it may be said to provide yet another example of the stirring truth that rabbits and human beings are very different that arguing from one to the other is fundamentally unsound and that time spent on the investigation of rabbits is so much time wasted, unless indeed the investigator should happen to be more interested in rabbits than in men.

The second attitude is to regard the difference as the starting point of an enquiry which by revealing the causes and significance of that difference, will enlarge our knowledge of both rabbits and human beings which will lead therefore, to a general theory of reparative processes to which human beings may or may not conform. An attempt to formulate such a theory now follows.*

A large defect in the full thickness of a freely

* The argument presented here follows closely the reasoning of Billingham and Medawar (2) see also Billingham and Russell a detailed quantitative analysis of the process of contraction (3)



FIG 44 Illustrating the epithelization of a raw area in a rabbit after applying a suspension of dissociated epidermal cells The greater part of the central epithelial island arose from five foci still identifiable in spite of their confluence Such a wound covering cannot prevent the process of contraction and is useless for permanent repair (compare also figure 43) A millimeter scale is attached (From a photograph by Dr R. E. Billingham.)

mobile region of a rabbit's integument heals by a combination of two distinct but partially concurrent processes, contraction and intercalary or intussusceptive growth. Contraction is a forced movement of the integument that brings about the closure of the wound by the apposition of its original edges. Intussusceptive growth is an enlargement of the skin in all three dimensions by the deposition of new skin elements upon the preexisting dermal framework. The one process closes the wound the other goes some way towards making good the actual loss of substance

Intussusceptive growth of skin is that which occurs in normal postnatal development and in a skin graft which transplanted to a young animal grows in step with its host. During wound healing in adults it is made particularly obvious by planting a skin graft (or leaving an island of undisturbed skin) in the center of a large raw area that undergoes contraction. When closure of the wound is complete the central graft or island may be as much as ten times its original area The hair follicles of the graft do not increase in number they simply move apart to a degree proportional to its linear enlargement.*

Evidently the pattern of the collagenous endoskeleton of the skin remains unchanged. Intussusceptive growth must be clearly distinguished from the increase of area that is brought about by the outgrowth of epithelium over the wound bed. Migratory outgrowth is an excursion of the epithelium beyond the perimeter of the original graft intussusceptive growth takes place entirely within its compass.

Contraction is an obvious enough process but the compensatory enlargement of skin is inconspicuous until the later stages of healing and is not likely to be seen unless it is looked for But two other contributions to the healing process are very conspicuous indeed the migratory ingrowth of epithelium from the margins of the skin defect (together with outgrowth from any graft or island of skin that may be contained within it) and the filling up of the wound bed with granulations that are slowly transformed into fibrous tissue. What is the significance of these?

If the reasoning of Billingham and Medawar (2) is correct, epithelium of outgrowth and the fibrous tissue of the wound bed are temporary organs of healing which make no substantive contribution to the state of final repair That they are temporary in wounds of the type now under discussion is certain for as contraction proceeds, it obliterates the space enclosed by the perimeter of the wound and the tissues that occupy the wound slowly disappear The function of the fibrous tissue (or rather of the fibrous transformation of the tissue of the wound bed) may well be as Lindquist has argued to provide the tensile forces responsible for contraction Lindquist s experiments show that contraction is due to a pull inwards from the wound bed and not to a sphincter-like contraction of the skin around the wound

The function of migratory epithelium is probably more complex. Epithelial ingrowth is faster than contraction—i.e the advancing fronts of ingrowing epithelium come together from opposite sides more quickly than the true skin edges from which the outgrowth takes origin so that the temporary closure of the wound by epithelium is complete before the final approximation of the true skin edges by contraction. The epithelium although unstable and ea y to peel away presumably seals what would otherwise be a raw wound bed agam t loss of body fluids from within and adventitious infection

* The existence of a phenomenon of hair neogenesis though now well established (4 5) does not affect the present argument

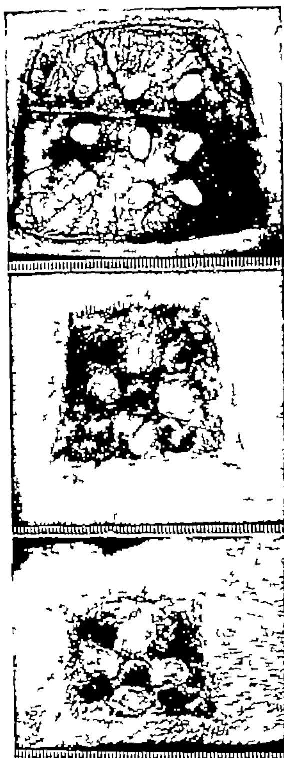


FIG 45 The breakdown of skin homografts in rabbits (*Top*) Nine pinch grafts have been symmetrically transplanted to a raw area prepared by removing the full thickness of the skin down to the level of the panniculus carnosus Four are autograft five are homografts. (*Center*) The same grafts ten days later The four autografts are healthy and well vascularized and each is surrounded by a halo of migratory epithelium The homografts are congested and hemorrhagic breakdown is far advanced Note contraction of the general wound bed (*Bottom*) The same grafts fourteen days after transplantation The homografts long since dead are now simply dry scabs Further contraction which will in fact proceed until the graft and wound margins come together completely (compare figure 43) A millimeter scale is attached to each photograph

from without. Billingham and Medawar attribute two other more important functions to migratory epithelium (*a*) to undermine and cause the sloughing away of dead dry tissue (eschar) and (*b*) to hasten the transformation of granulation tissue into fibrous tissue.

Healing without a scab is an artifact of surgical care. A tough and tenacious scab would be a serious impediment to natural healing were it not for the spectacular power of skin epithelium to cleave between living tissue and dead This function is not exercised in wounds which heal under surgical ministration and is therefore usually forgotten.

Evidence that the presence of an epithelial cover hastens the transformation of granulation tissue into fibrous tissue is given by Gillman and coworkers (6) Its action, we have found is particularly conspicuous where the growth of granulations elsewhere has been retarded by the administration of cortisone. If the maturation of fibrous tissue in the wound bed provides the tensile force of wound contraction it follows that epithelial cover by hastening the formation of fibrous tissue should in turn hasten contraction.* So far it has only been shown that the presence of an epithelial cover does not *retard* contraction.

The foregoing argument (founded it must be emphasized, upon the study of wound healing in *a highly mobile integument*) amounts to this. Contraction not epithelial ingrowth is the mechanism of definitive wound closure The loss of skin entailed by wounding is at least partly perhaps entirely made good by an intussusceptive growth upon the framework of the residual undamaged skin. The migration of epithelium over the wound bed and the fibrous transformation of the granulation tissue within it make no substantive contribution to final

* Recent work in the Department of Anatomy of University College London (7) has now thrown grave doubts on the widely held belief that contraction is caused by the maturation of young collagen fibers The contractile element may be the fibroblast cell

that the patch grafts enlarge by intussusceptive growth under the tensile forces of contraction and so end up by being worth *more* than their original value of true skin. The enlargement of patch grafts by true dermal growth under tension has been proved to occur in rabbits (see above) if it also occurs in human beings (and it is to be hoped that plastic surgeons will try to find out whether or not it does) the case for the theoretic merits of patch grafting will be firmly established.

*All the solutions discussed above started from* the presumption that only the patient's own skin could be used for repair The remainder of this chapter is concerned with the possibility of using grafts from other donors

## HOMOGRAFTS THE GENERAL PROBLEM

It is no exaggeration to say that surgery would enter into a new dimension of accomplishment if tissues could be transplanted with the same freedom between two different individuals as between one part and another of a single individual The present situation is that homografts of all tissues in all positions of the body must be presumed to be impermanent until the contrary *has been clearly proved.*

Let us first ask if there is any *biologic* sense in the homograft reaction, or more generally in the sort of diversity of antigenic make-up which *leads to incompatibilities of transfused blood* and of grafted tissues. In other words is there some underlying good reason which makes the homograft reaction understandable and biologically apt? This is an important question if the answer is in the affirmative it may follow that the surgeons' attempt to break down the natural barrier to homoplastic transplantation is a zoological indiscretion if not something worse

Fortunately there are no grounds for alarm. Geneticists are generally agreed that inborn diversity as such is a good thing —roughly speaking because it governs the versatility of the response of an animal population to the forces that are responsible for evolutionary change. There is no reason to doubt that antigenic diversity with all that it entails, is simply a tiresome by-product of this otherwise praiseworthy genetic inequality for the property of being antigenic is, after all, one that only reveals itself in the unnatural context provided by the operations of transfusion and grafting.

So far as our knowledge goes, there is no harm in the permanent survival of a living homograft, i e no harm in an animal's being a mosaic or chimera of tissues that belong by right of embryological origin to two or more different individuals.* By methods to be described later permanently successful homografts can be transplanted in mammals and birds and their hosts are *in no perceptible way at a disadvantage.* These 'tolerant' hosts are of artificial origin Chimeras of natural origin in higher animals were unknown until 1945 when R. D Owen *showed that most twin* cattle contain a mixture of each other's red blood corpuscles and erythropoietic cells We discovered later that the majority of twin cattle are mutually tolerant of grafts of each other's skin so that an artificial chimerism of skin could be superimposed upon the preexisting red cell chimerism of natural origin.†

The chimerism of twin cattle is associated with a serious and peculiar infirmity with most twin cattle of unlike sex, the female (a 'freemartin') is to various degrees infertile There is an exact correlation between the occurrence of chimerism and of infertility and also between chimerism and that modification of immunologic response which causes twin cattle to become tolerant of grafts of each other's skin. At first sight these correlations are most ominous the one natural example of the breakdown of the *barrier to homografting is associated with a* major biologic disability

Closer investigation shows that these misgivings can be set at rest. The fact that in cattle *red cell chimerism, tolerance of homografts and* in the females of twins of unlike sex, infertility all go together is due to their sharing a single anatomical prerequisite in common *viz.* the synchorial condition of the embryos in consequence of which there is an interchange of fetal blood. But the association is by no means obligatory We find that tolerance of homografts

* The special circumstances (8) under which a state of chimerism can be harmful are discussed later (Reaction of Graft against Host) The chimerism discussed in the present section is that which occurs naturally in dizygotic twins and it leads to a state of *mutual* tolerance from which no evil consequences need be expected

† *One case of natural chimerism* in human beings was recorded in 1953 (9) and two other pairs have been described since Chimerism may also occur in sheep (10) and it is apparently the regular thing in twin chickens (11)

from male animals in females can be achieved without any interference with reproductive function. The three properties go together in cattle because of the common ancestry of the pedigree of causes, not because tolerance or chimerism is responsible for freemartinism.

The surgeon therefore has no reason to fear that aspiring to use homografts for permanent repair entails brushing aside some prudent natural safeguard against mixing tissues of different genetic origins. The *failure* of a homograft is more to be feared than its success. A "toxic syndrome" has been spoken of in connection with the rejection or dissolution of unaccepted homografts, and with organs of the size and position of homografted kidneys it must, as Dempster points out, be taken seriously. Gorer has warned us of the possibility that the rejection of a tissue homograft may cause the formation of red cell agglutinins, which in certain circumstances could prejudice the outcome *of a later transfusion or pregnancy. This danger* is avoidable. More generally, the idea of having tissue in the body which is undergoing aseptic destruction or autolysis is surgically offensive, although there is no very definite evidence of its *doing* serious harm.

A homograft's expectation of life after transplantation varies with (amongst other things) the tissue that is grafted and the position of the body into which it is put. It is hardly to be supposed that these variations are capricious or unsystematic. The writer's present opinion is that *one* fundamental system of genetic and immunologic laws governs the behavior of all kinds of homografts, no matter where they may be transplanted, and that these laws are most harshly enforced in respect of orthotopic homografts of skin. In other words, the orthotopic *skin homograft is the* one which under normal circumstances can be relied upon most confidently *to fail. The* partial or complete success of homografts of other tissues, or of skin homografts transplanted into positions other than skin, is due to their dispensation from or avoidance of one or more of the laws of transplantation—dispensations which are sometimes intelligible and sometimes not, but which are in any event to be thought of as privileges and not as *rights. Thus* it will be suggested that corneal homografts survive because they are in a position of the body to which the agencies of destruction cannot gain access; that homografts in the brain survive for long periods because in that position the antigenic stimulus can elicit no response; that bone and arterial homografts may "work," in the sense of being clinically successful because they are, in Longmire's terminology (see above), "homostatic." Endocrine homografts sometimes succeed for reasons which, if not entirely obscure, are at present far from clear. Homografts between identical twins behave exactly like autografts: such twins begin life as a single individual and for genetic and immunological purposes can be assumed to remain so. Homografts between dizygotic twin cattle are accepted because of the mutual suppression of their powers of immunologic response (see below, Actively Acquired Tolerance); homografts of rapidly growing tumors can overcome a feeble immunologic opposition in a way that is denied to skin. In all such cases it is survival or success that has to be explained away in the face of a *prima facie* expectation of failure. It will be as well therefore to base our *exposition upon the behavior of orthotopic* homografts of skin, and then to discuss the different ways in which other tissues have been found to escape their fate, but before this can be embarked upon, it is of particular importance to discuss the genetic laws that govern the acceptability of homoplastic grafts.

## GENETIC LAWS OF TRANSPLANTATION

When every other factor that may influence a homograft's expectation of life has been brought under control—the species of choice, the nature and size of the graft, the position of the body into which it is put and the way *it is put there*, the reactivity of the host (in so far as it may be influenced by states of stress or other peculiarities of endocrine function) and the host's *previous* experience of grafting from the same or kindred sources—when every such factor has been brought under control, there still remains a source of variation which outweighs all others, *viz.*, the genetic relationship between the donor and the recipient.

One possible misunderstanding *of the ambit* of the genetic laws of *transplantation must be* cleared up at the very *outset. If* homografts are transplanted to regions of the body in which they can elicit no reaction from their hosts—in *which, therefore,* they survive and flourish—then they will stand outside the genetic laws of transplantation and cannot be influenced by them. To maintain, as more than one investiga-

tor has done that the genetic laws are illusory because even heteroplastic grafts may flourish after transplantation to the brain is on all fours with saying that Criminal Law is non-existent because people who commit undiscovered crimes are not sent to jail.

The surgeon is inclined to deprecate (and may well deprecate) the importance of the genetic influence on the behavior of homografts because in clinical practice there is very little he can do about it. His patients are "given," and not, as in experimental science discretionary variables. He has some power of discretion in the choice of a donor of homografts but very few rules by which to exercise it. There is at present no "typing" of tissue donors and (as Woodruff has clearly shown) no evidence that compatibility of a donor's blood with a recipient's makes much difference to the fate of homografts of ordinary tissues. If he chooses a relative as donor it is as likely to be for reasons of general expediency as for genetic relatedness—and this is just as well, for pedigree relationships of the kind that were at one time embodied in the term *syngenesiotransplantation* can be a most misleading guide to genetic affinities.

That we now have a very clear understanding of the genetic basis of transplantation is due to the long systematic researches at the Roscoe B Jackson Memorial Laboratory at Bar Harbor under the leadership of C C Little and latterly of G D Snell reinforced by the genetic and immunologic studies of P A. Gorer at Guy's Hospital, London. Most of this work has been done with homografts of tumors and (of necessity) with mice but there is no reason to doubt that its principles apply to homografts of other kinds and to other species of animals. The work has not received the attention from surgeons that it deserves. Most surgeons and many biologists have been put off by the complexities of genetic notation combined with a stolid indifference to the genetic theory of transplantation *as such*. It is hoped that the summary which follows will give the gist of the information that is necessary if experiments in transplantation immunity are to be of effective design.

The facts which the geneticist assembles are in the form of records of the success or failure of grafts of known origin in animals of known genetic provenance. "Success" with tumor homografts usually means the progressive growth of an implanted tumor fragment, leading to the death of its host "failure" usually means regression after a short period of growth or even failure to grow at all. The scoring of success and failure will of course vary with the criteria that are adopted and with the properties of the tissue that is used. A degree of compatibility that allows a tumor homograft to grow in an alien host can often be shown to be quite insufficient to allow the normal healing and differentiation of a homograft of skin But this is not, as it is sometimes thought to be an important objection to the fundamental techniques of genetic analysis. All scoring systems are to some extent arbitrary what is vitally necessary is that they should be capable of being consistently applied.

By adopting any one system of scoring it is found that homografts are uniformly successful when transplanted between members of a highly inbred strain and uniformly unsuccessful when transplanted between members of two different strains. (By different strains must be understood two strains of distant common ancestry) A "within-strain" homograft is therefore, in effect, an autograft a "between-strain" homograft is a "true" homograft in the sense in which a graft between any two human beings, or any two rabbits from a heterogeneous stock, can be so described *

When members of two inbred strains are crossed, the hybrid offspring are found to accept homografts from each other from members of either parental strain or indeed from the immediate or remote progeny of crossing two such hybrids. The first hybrid generation ($F_1$) is therefore a "universal recipient" for an assembly of individuals comprising the two parental inbred strains and the immediate or remote issue of their crosses.

This relationship is not reciprocal. Homografts taken from the $F_1$ progeny will be rejected by all members of the parental strains. They will be rejected by some at least (generally a great majority) of the $F_2$ progeny produced by mating two $F_1$ hybrids or of the $B_1$ progeny produced by backcrossing one of the $F_1$ hybrids to a mem-

* "Within-strain" homografts are sometimes described as "isografts" There is one most important exception to the rule that isografts are normally successful Eichwald and Silmser (12) find that in some strains of mice females will not permanently accept skin grafts from males a fact which has since been confirmed in several other laboratories

ber of one of the parental strains Correspondingly grafts from members of either of the parental inbred strains though they behave as if they were autografts when transplanted to $F_1$ hybrids will only be accepted by a proportion (generally a very small proportion) of the $F_2$ or $R_1$ generation

The fact that individual differences of the type that influence the outcome of homografting are extinguished by inbreeding shows that these differences have a genetic origin, and are not due to the fact that the different individuals are necessarily reared in somewhat different environments. Further, the fact that there is a *segregation* in the $F_2$ and $R_1$ generations of the power to accept or reject homografts of parental origin reveals the working of a particulate or Mendelian scheme of inheritance.

The actual experimental findings are accommodated by the following hypothesis. The failure of a homograft depends upon the presence within the grafted tissue of one or more antigenic factors absent in the host. The presence of each antigen is determined by the presence of a single Mendelian factor or allele. The genes are dominant in the rather special sense that (like the genes determining the presence of the human blood group antigens A and B or M and N) they make their presence felt in the heterozygous as well as the homozygous state that is, whether or not the allele happens to be paired in the chromosomes with another similar to itself Homografts can be successfully exchanged between the members of a single highly inbred line because inbreeding has extinguished genetic variance and the members can be regarded as identical and homozygous for all the relevant genes. The $F_1$ hybrid generation is also a uniform population in the sense that its members are genetically alike but according to Mendelian rules it will be heterozygous at every gene locus in respect of which the two parental strains differ It follows that each of the $F_1$ progeny contains at least one representative of every gene present in either parental strain The $F_1$ animals are therefore universal recipients

The greater the number of antigen controlling genes by which the two parental strains differ the smaller will be the proportion of successful homografts when tissues from (or tumors indigenous to) the parental strains are grafted to the $F_2$ or $R_1$ progeny By making the simplest possible assumptions it follows from elementary Mendelian rules that the proportion of compatible hosts will be $(3/4)$ in the $F_2$ and $(1/2)$ in the $R_1$ generations where the parental strain chosen to provide the graft donors differs at n antigenic loci from the inbred strain with which it was crossed.

In practice it turns out that two inbred strains may differ by upwards of a dozen genes controlling antigens which influence the outcome of homografting in a conspicuous way * and in all probability by a still larger number of antigens which are weak in the sense that their effects will be revealed only by using a rather sensitive system of scoring for success or failure.

The practical import of the genetic rules of transplantation is quite simple. Every experiment on transplantation immunity should be genetically standardized whenever possible. When genetic standardization is not possible one should be at all times consciously aware of the fact and make allowance for the errors which it must necessarily introduce. 'Genetic standardisation need mean no more than the carrying out of experiments on animals of known genetic origins for example, by choosing the donors of homografts from one inbred strain and their recipients from another It is very difficult to envisage any experiment in which the use of heterogeneous animals would be an advantage, unless the experiment happened to be expressly designed to reveal the extraordinary variety of responses of which a heterogeneous assembly is capable (and it might be added, the extraordinarily confusing results to which their use can lead) Only when genetic standardization is impossible will it be advisable to choose donors and recipients at random from highly heterogeneous stocks In short, uniformity is desirable and *random non-uniformity* is acceptable between the two is a no man s land in which many experiments have come to grief

One cautionary example may be given of the dire consequences of failing to make due allowance for the errors introduced by an absence

* The more recent work of Snell Gorer and their colleagues (13 21) shows that one very important system of antigenic differences in mice is governed by a complex of upwards of ten alleles lying on the ninth chromosome in the neighborhood of certain marker genes affecting the development of the tail An important recent study of the number of loci affecting the survival of skin homografts in mice is that of Barnes and Krohn (31)

of genetic standardization. It was for a long time believed that the regression or rejection of a homograft did not in any wise heighten its host's resistance to a second homograft transplanted on a later occasion. The experiment upon which this important negative finding was based consisted of transplanting a second-stage homograft from a donor other than that which provided the first homograft. The animals used in the experiment were of a heterogeneous stock inasmuch as the antigenic make-up of the second donor could perfectly well have been very different from that of the first there is no particular reason why the immunity produced by the first homograft should have been visited upon the second The *same* donor should have been used for both graftings alternatively had it been possible, the donors of both homografts should have been members of the same highly inbred strain.

Very much more is now known of the genetics of tissue transplantation than the foregoing summary may suggest nor has the newer knowledge always led to greater simplicity The complications that arise are no more discreditable to genetic theory than was the discovery of isotopes to elementary chemistry or of irrational numbers to mathematics but they should not be concealed as Pythagoras is said to have concealed from the world at large the painful truth that not all quantities are expressible as ratios of simple integers

Some of these complications concern inbred strains Some species or stocks of animals of which the laboratory rat might possibly be one resist the theoretic approach to complete genetic uniformity on inbreeding and can never be assumed to form an entirely homogeneous population Even with mice the genetic uniformity of an inbred strain should be treated as a probationary assumption and not as an article of faith. Certainly an inbred strain divided into sublines will diverge in respect of properties that influence the outcome of grafting and, if sensitive tests are used incompatibilities can be revealed by the exchange of homografts between members of sublines which stand as few as a dozen generations apart. (It is for this reason that geneticists annotate their domestic sublines of well-known strain by differential symbols, the use of which is one of the major bugbears of genetic prose) The divergence of sublines may be due either to the unmasking of heterogeneity within the common parental line or to the origin of new variants (mutations) since their separation.

Second, the fate of normal tissue homografts is probably influenced by a large number of genes controlling antigens which are severally of minor effect and their effects may be additive when they act in unison the genes may differ moreover in their degree of 'penetrance' which roughly speaking means their power to take effect. It follows that the crisp patterns of segregation to be expected when only 'major genes' influence the outcome of grafting become blurred, and the pattern of segregation tends towards the quantitative form of polygenic inheritance rather than that of multifactorial inheritance

The two reservations mentioned above should put one on guard against an unduly simple interpretation of the genetic laws Two others direct attention to loopholes in their enforcement.

Antigenic constitution is usually thought to be as deeply innate or inborn as any endowment can be—to be determined genetically and impregnable to environmental interference Owen's discovery of natural graft hybrids in dizygotic twin cattle (see above) in which each twin has its partner's blood-antigens superimposed upon its own, shows that there are exceptions to this rule, although perfectly intelligible ones. Inasmuch as one of the consequences of chimerism is to abolish a twin's reaction to grafts of its partner's tissues it follows that the genetic laws of transplantation stand in abeyance for this particular case. There should also be mentioned Dr Barrett's discovery (14) of strange alterations in the transplantability of tumor homografts which follow even quite a short residence in an apparently compatible environment. These aberrations are not yet fully explained at present it is only necessary to put on record the fact that they exist.* It is unlikely that they will prove to have an important bearing on the use of homografts in clinical practice.

## IMMUNOLOGY OF HOMOPLASTIC TRANSPLANTATION

### Natural or Acquired Immunity?

*The concept of an inborn or ready made immunity against tissue homograft* has never

* For a recent discussion of this remarkable and important phenomenon see Klein and Klein (15)

been proposed with much conviction and cannot in any event be sustained. A homograft transplanted in a normal animal enjoys an immunologic latent period during which its healing growth and behavior fail to distinguish it in any perceptible way from an autograft of the same kind.* The length of the latent period depends above all else upon the immunologic disparity between donor and host—in effect, therefore upon their genetic relationship. At one extreme skin homografts transplanted between rather closely related mice (e.g., from a male to a female of the same inbred strain) may remain perfectly normal to outward appearance for more than a month during which period they will regenerate a normal pelt of hairs and become fully incorporated into the skin of the host. At the other extreme skin homografts transplanted between rabbits or cattle of different breeds may be destroyed in an acute inflammatory convulsion within seven days of transplantation. Other factors that influence the length of the latent period include the "dosage" variable (i.e., the quantity of foreign tissue to which the host is exposed) and the immunologic reactivity of the host (as it may be influenced, for example, by the secretions of the adrenal cortex). These will be mentioned in due course below.

## Antigenic Stimulus and Site of Primary Reaction

A substance (or a complex of substances) may be described as "antigenic" if, having been given a fair opportunity to do so, it elicits an immunologic response from the organism into which it has been introduced. Being thus defined by performance and not by pretensions, the adjective "antigenic" is clear enough. The noun "antigen" is, however, very vague. It is useless to attempt to define the substantive properties that make a certain molecule an antigen in its own right, for the property of being an antigen (like that of being a husband or being a nuisance) is entirely relative or contextual. The nearest one can get to "defining" an antigen is to list certain properties lacking which no substance is likely to be antigenic under any circumstances; but even the least demanding of these properties—

* Recent work on goldfish by Dr. W. H. Hildemann (T.) at the California Institute of Technology has shown that the latent period during which autografts and homografts are indistinguishable may be very short.

that of having a molecular weight not less than 10 000 or thereabouts—needs a number of saving clauses before it can be applied to simple chemical substances like picryl chloride or dinitrochlorobenzene which, aptly administered, can produce skin sensitivity of the delayed type.

Of what nature are the antigens that cause transplantation immunity? This is a question that has defied answer for more than fifty years—mainly because the antigens are grievously unstable, so that even the most humane methods of killing cells usually deprive them of the power to incite a reaction. Genetic evidence has made it clear that the "transplantation antigens" characteristic of any one individual are numerous and under exact genetic control, but the identification and labelling of antigens by genetic methods make no assumptions about, and give us no information about, what the antigens actually are. Nevertheless it appears (a) that the power of living whole blood to incite a homograft reaction resides in its leukocytic fraction and is not to be found in plasma, red cells or blood platelets, and (b) that all the antigens characteristic of any one individual are to be found in all the viable nucleated tissues of the body. The grounds for this second belief will be given later. The antigens we are seeking are evidently to be found only in nucleated cells, but, apparently, in all nucleated cells.

My colleagues and I (16, 34) have recently described methods by which cells can be totally disintegrated *without* destroying their power to elicit transplantation immunity, and it has been found that the antigenic substances are specially concentrated in the nuclear fraction of disintegrated cells. Their solubility and their behavior under treatment by specific enzymes suggested that the antigens were desoxyribonucleoproteins, but more recent evidence (34) points rather towards a determinant group of mucoid nature, chemically akin to the human blood group substances.

That the regional lymph nodes are the primary seat of the homograft reaction has now been proved beyond all reasonable doubt. Thanks to the use of an ingenious experimental design, Mitchison (18) has been able to prove *that a state of sensitivity, immunity, or heightened resistance to tumor homografts can be transferred from one host to another by cells taken from the regional nodes.* My colleagues and I, working with homografts of skin, have confirmed Mitchi-

son's work in full, adding to it the fact that the spleen may also participate in a quantitatively rather feeble way (33)

In its simplest form an experiment demonstrating passive or (as we prefer to call it) "adoptive" immunization has the following design. Three animals are used a donor D and a primary and secondary host, $R_1$ and $R_2$ respectively Animals $R_1$ and $R_2$ must be of the same strain, so that tissues transplanted between them provoke no reaction on their own account the donor D must be of a different strain. A homograft is transplanted from D to $R_1$ and its survival time (if not already known) recorded When the reaction against D-tissue is at its height, the regional lymph nodes of $R_1$ are removed and cells expressed from them are inoculated *intraperitoneally into* $R_2$ The secondary host $R_2$ is then challenged with a homograft from D If a state of immunity has been transferred, the homograft transplanted from D to $R_2$ behaves *as if* $R_2$ had been actively immunized beforehand its survival time is much shorter than that of the graft transplanted from D to $R_1$ The cells of $R_1$ which have this power to transfer a state of heightened resistance are preeminently those of the regional lymph nodes serum and blood or exudative leukocytes are ineffective, and the power of cells from regional nodes to transfer immunity is abolished by subjecting them to any treatment that kills them. Very careful studies give satisfactory evidence that adoptive immunity can be attributed neither to a passive transfer of preformed antibodies nor to the active immunization of the secondary host by antigenic matter unwittingly transferred in the lymph nodes taken from the primary host.

Mitchison's work on the establishment of 'adaptive immunity' by transferring immunologically active tissue from homografted to normal hosts is perhaps the most important single proof of the immunologic nature of the homograft reaction. To round off the story it should be added that two independent groups of workers have shown that the regional lymph nodes when confronted with the stimuli arising from a tissue homograft, undergo just that sequence of histologic and cytologic changes which is associated with antibody formation in more orthodox immunologic systems (17)

This is the proper place to mention a certain apparent paradox about the tissue antigens no one of them, singly considered, is essential for the life of the organism or the cell. This is not *an empirical finding* but a tautology A tissue antigen responsible for transplantation immunity can be recognized only if there exists at least one organism in which it is absent, and to which it is therefore inessential. If it were present in all the members of a species, the putative antigen would have no opportunity to reveal itself for it could never provoke immunity Thus, singly considered all the substances that act as antigens must be of minor metabolic importance, or at least dispensable. The argument is exactly the same in principle as that which explains away the *relative* unimportance of the effect of most Mendelian genes in the face of the ill informed and far from kindly meant criticism that geneticists are preoccupied with the study of trivialities.

## Specificity

The destruction of a tissue homograft leaves its host in a refractory state, or state of heightened resistance which lasts for many months.* A second homograft from the same donor transplanted within this period is destroyed more rapidly than its predecessor and to the accompaniment of a very much modified vascular and inflammatory response. The state of heightened resistance is systemic in compass it is not specially confined to nor even specially effective within the position of the body in which the first homograft was destroyed. This is only to be expected if lymph nodes are indeed the instruments of the host's response to regional homografts, *for the efferent pathways from lymph* nodes have direct access to the systemic circulation

*The state of sensitivity* provoked by homografts from one donor will visit itself upon the tissues of a second donor if and only if the two donors share antigens which are not possessed by their recipient. This theorem has been established with complete precision by the experiments in

* The 'lifelong immunity' that is often spoken of in connection with homografts in mice applies only to the immunity that is provoked by homografts of solid tissues and its duration may be connected with the local cellular response We have recently found that the sensitivity provoked by the intraperitoneal injection of up to a million dissociated homologous spleen cells is quick to arise and quick to decay

which Dr G D Snell has transplanted homografts between members of inbred strains of mice that differ by single antigen-determining genes. There is then, an "individual specificity" in the homograft reaction. But, so far as present information goes, tissue specificity or embryologic specificity is altogether lacking The evidence for this important generalization comes not, as might be expected from the study of cross-reactions in acquired immunity but from the study of cross-reactions in acquired tolerance. It is true that the injection of adult animals with blood leukocytes will immunize them against homografts of skin epithelium from the same donor but this experiment (and others of the same design) shows nothing more than that skin epithelium and blood leukocytes have antigens in common. It is very much more to the point that the injection of very young animals with blood leukocytes can confer *tolerance* of homografts of skin and this can only mean that skin epithelium contains *no* antigen that is not also present in the leukocyte.

Other combinations of tissues tell the same story Woodruff and Sparrow find that spleen homografts transplanted to newborn rats will cause their hosts to accept thyroid homografts in later life so also P S Russell and I have shown that mice injected immediately after birth with splenic cells are tolerant of homografts of adrenocortical tissue. If adrenocortical tissue contained "transplantation antigens" that were not present in the same donor's splenic cells, then the injection of splenic cells could not confer tolerance of adrenal homografts How could they cause tolerance in respect of antigens which they do not possess? In short, an individual's antigenic constitution is a trade mark stamped upon all his cells or at least upon all his viable nucleated cells. This inference is in no way at odds with the well known fact that the tissues of a single individual may differ very widely in respect of the antigens that may be revealed by "heterologous" immunity reactions—*e.g.*, by injecting the tissues of mice into rabbits—or in respect of the antigens that can be induced to form "auto-antibodies" *i.e.*, that can provoke immunologic reactions from the individuals of which they themselves form a part.

### Antibodies

The homograft reaction belongs to that *demi monde* of immunologic responses in which "classical" antibodies seem to play no necessary part. This is not a proposition that would be accepted by all students of transplantation immunity for as P A. Gorer was the first to show the transplantation of homografts does certainly incite the formation of orthodox serum antibodies—antibodies which may be recognized not always easily by their power to agglutinate the red blood cells or leukocytes of the donor The fact that homografts *can* cause antibodies to be formed is not in question nor, in the face of evidence from Gorer Kidd Burmester Billingham and Sparrow, and many others can it be denied that under special circumstances these antibodies will exercise a cytotoxic action. The problem is whether or not serum antibodies *do* play any part in the regression of orthotopic homografts of normal tissues. Present evidence suggests that they do not.

For example it is not normally possible to secure a true passive transference of transplantation immunity by means of serum. The times at which transplantation immunity and serum antibody formation are at their peak are clearly different (18) Homografts may be rejected by fetal lambs (19) at an age before they have acquired the power to manufacture gamma globulins *i.e* to manufacture proteins of the class to which most antibodies belong fetal ungulates are agammaglobulinemic. The presence of a high titer of serum antibodies is often associated with a weakening of the homograft reaction—with the phenomenon of "enhancement," to be mentioned again below

Finally, certain experiments of ingenious design carried out at the National Cancer Institute (20) seem in themselves to unseat the hypothesis that serum antibodies, in the concentrations at which they actually occur in animals which are demonstrably sensitive or immune play any necessary part in destroying homografts. Homografts enclosed within porous chambers were inserted into mice which had been sensitized beforehand against the donor's tissues. The pores were of alternative sizes large enough to let through cells, or too small to let through anything except body fluid and matter dissolved in it. The homografts were destroyed if and only if the pores were large enough to give passage to host cells. Other variants of this experimental design give still further weight to the inference that serum antibodies play no necessary part in the homograft reaction.

This rather peremptory discussion may well

have done less than justice to the brilliant research which has led to the immunologic and genetic labelling of the iso-antibodies formed after the transplantation of foreign homologous cells the balance may be redressed by consulting the fuller discussion by Gorer (21)

**Reaction of Graft Against Host**

Dr W J Dempster was the first to call our attention to the fact that a homograft containing immunologically reactive tissues is theoretically empowered to react against its host. There is clear histological evidence of such a reaction in homografts of whole kidneys in dogs. Yet it would be unwise to make too much of it. For example $F_1$ mice—mice which are the immediate progeny of a cross between members of two inbred strains—will accept without apparent distress, homografts of skin or tumor from any member of the parental strains. Yet the host certainly contains antigens not present in the graft it contains, to be exact all the antigens contributed by the parent which was not the donor of the graft.

There is however one well defined circumstance under which a graft-against-host reaction may be damaging to the point of being fatal when newborn mice (and probably other animals too at an equivalent stage of development) are injected with immunologically competent cells from donors belonging to genetically distant strains (8) For example newborn A line mice will die within a few weeks of the injection of splenic or lymph node cells from donors of strains AU or C57 The mechanism seems, in a first approximation, to be simply this the newborn host is not so old that it cannot become tolerant of the injected cells, which can indeed be shown to survive and flourish so long as their recipient remains alive but the injected cells (being adult and of the kind that undertake immunologic responses) react upon and destroy their hosts When the severity of the graft against host reaction falls short of being fatal, its victims grow up weak and stunted and with a generalized lymphoid hypoplasia The very searching analysis of this runt disease by Billingham and Brent (8) has made it clear beyond all doubt that it is an *immunologic* disease even though the immediate cause of death is still uncertain Runt disease is not caused by the injection of foreign embryonic cells or adult hybrid cells there is therefore no causal connection between tolerance and lymphoid hypoplasia Exactly the same is true of the gross splenic enlargement produced by the injection of chicken lymphoid cells into embryonic or newborn chicks a phenomenon shown quite independently by Simonsen (35) to be due to a reaction of the grafted cells against their host.

The most important theoretic consequence of the study of runt disease and kindred phenomena is that it provides a new method of identifying immunologically competent cells i.e. cells qualified to engage in immunologic reactions Because of its power to cause runt disease it is certain that some cell present in the leukocyte fraction of peripheral blood is immunologically competent The surgical import of graft-against-host reactions is rather grave The successful homografting of a complex organ like the kidney may not depend merely upon the abrogation of the host's reaction against the graft it may also be necessary to weaken or abolish the graft's reaction against the host. Research upon this problem is urgently needed for no one yet knows whether a graft-against-host reaction is sufficient *in itself* to destroy a kidney homograft or to stop it working Caution should also be exercised in the grafting of lymphoid tissues themselves, particularly in children.

**Special Dispensations**

An orthotopic homograft of skin, transplanted between two normal and genetically unrelated *adult mammals or birds never survives* "Never" is perhaps an inductive heresy * certainly there is no theoretic reason why luck of sampling should not occasionally present us with a donor of grafts containing no antigen not also present in the recipient and the odds against it so Dr I J Good has reminded us are far from astronomic But the present writer and his colleagues between them, have transplanted skin grafts between many thousand rabbits of heterogeneous outbred stocks and have never found a homograft to endure. When donors and recipients are of known and virtually constant genetic disparity as with mice of two different inbred strain then the indefinite survival of a homograft—depending, as it would upon the simultaneous occurrence of upwards of twenty mutations at histocompatibil-

* And is shown to be so by the remarkable fact discovered in the Department of Biology in Boston University that homografts in hamsters last much longer than in any other animal yet studied (23)

ity loci and therefore upon odds in the neighbor hood of 50 000[20] 1—could be expected to occur only in work carried out with the collusion of Maxwell s Demon

But some kinds of homografts do nevertheless endure. The reasons why they do so may be classified under three headings (a) failure to provoke immunity (b) inability to respond to a state of immunity and (c) survival for reasons still unknown.

(a) *Failure to provoke immunity* A homograft may fail to provoke immunity because it has been transplanted to a region of the body in which its antigenic propensities cannot take effect. One such region as Murphy showed long ago and many others have since confirmed is the substance of the brain But a homograft will not survive in the cerebral cortex if the host has been immunized beforehand by a graft in a position of known antigenic effectiveness elsewhere. The survival of homografts in the brain is therefore presumably due to their failure to elicit an immune response not to their being inaccessible to its consequences. Recent work which has shown the regional lymph nodes to be the anatomical seat of the immunological response against regional homografts rather favors the view that homografts survive in the brain because of its lack of an ordinary lymphatic drainage system So far as the writer is aware, no attempt has ever been made in clinical practice to use the brain as a site for the transplantation of for example homografts of the parathyroid gland nor has a systematic search been made for other immunologically privileged positions elsewhere.

The successful use of homografts of segments of blood vessels must be attributed to the fact that their functional effectiveness does not depend upon their being alive. Frozen-dried and reconstituted vascular homografts do equally well and these either have lost their antigenic power or cannot exercise it. The successful use of plastic replacements suggests that the useful part of a vascular homograft is its fibrous endoskeleton It is not or need not be a living homograft at all.

(b) *Inability to respond to a state of immunity* The most merciful dispensation from the rules of tran plantation immunity is that which is enjoyed by homografts of the cornea There is nothing immunologically anomalous about corneal tissue as such Billingham and Boswell have shown that transplanted heterotopically to an area formerly occupied by skin a corneal homograft behaves just as a skin homograft does. What is distinctive of the corneal homograft is its anatomic situation. Billingham and Boswell found that a skin homograft transplanted into the cornea will survive even in a host which has been strongly immunized beforehand by skin homografts from the same donor in an orthodox position on the recipient s chest. This pattern of behavior is apparently the converse of that which obtains in the brain homografts in the brain endure because they cannot provoke immunity and in the cornea because they cannot respond to it. But the homograft in the cornea only survives in an immunized host if it remains avascular and the same is true in the writer s experience of skin homografts transplanted into the anterior chamber of the eye Inaccessibility to an immune reaction is then a *sufficient* explanation of the success of orthotopically transplanted corneal homografts It is not the only possible explanation and it may not be the most important. If as Maumenee has suggested the corneal homograft is partly replaced by tissues of the host, and if the anterior chamber of the eye the intermediary in its metabolic activities is in any case a position of the body in which the immunizing power of a homograft is somewhat feeble then a homograft problem may not arise in an acute form at all

Homografts of living cartilage may survive not only in an immunized host, but even in the midst of the inflammatory convulsion associated with the destruction of other homografted tissue in the immediate neighborhood Although a number of perhaps unduly profound meanings have been read into this fact the writer is not yet satisfied that we need look beyond the avascular state of a cartilaginous graft *for an explanation* Like the corneal homograft it has a *kind of* extraterritorial status as a tissue culture *in vivo* More will be said later of the *part played by blood* vessels in the *fulfilment* of the homograft reaction at present the correlation between an avascular *state* in a homograft and its anomalou survival will be assumed to stand

(c) *Survival for reasons unknown. the endocrine homograft.* It has been mischievously said that *homografts* of endocrine tissue always used to succeed until endocrinologists began to learn of excellent theoretic reasons why they might be expected to *fail.* Most of the earlier litera-

endocrine homografting is so poorly documented from an immunologic standpoint that little can be made of it we do not know whether its authors exchanged homografts between inbred or outbred animals or between the members of closed (and therefore partly inbred) colonies in which a certain proportion of homografts might in any event have been expected to succeed. It is nevertheless clear from the evidence summarized in Dr P L. Krohn's chapter in this volume that homografts of endocrine tissue do *in the main* conform to the rules of transplantation immunity but it is equally clear that, unless a very large number of competent endocrinologists are the victims of a conspiracy of mischances, homografts of endocrine tissue do *sometimes* most anomalously succeed In their very careful study of the fate of subcutaneous ovarian homografts in rats Harris and Eakin did indeed find that the proportion of successes fell away rapidly as the genetic gap between donors and recipients widened but for all that, between 25 and 30 per cent of true (interstrain) homografts in oöphorectomized recipients gave evidence of functional survival after one month, and a number were still surviving after three months. The later work of Lehrfeld and Taylor justifies the retrospective guess that the median survival time of skin homografts exchanged between rats so distantly related would have been about 10 days, and that not one would have survived so long as 30 days.

That ovarian homografts in oöphorectomized rats enjoy some privilege withheld from homografts of skin is shown very clearly by the recent work of Billingham and Parkes (24) Using groups of individuals sampled at random from a single closed and partially inbred colony of white rats Billingham and Parkes found that 17 out of 31 (55 per cent) of animals rejected their skin homografts within three weeks of transplantation whereas in a separate but otherwise entirely comparable sample, 16 out of 19 (84 per cent) of the recipients of subcutaneous ovarian grafts were still displaying cycles of vaginal cornification after two months It is a most significant fact however that if the recipients of ovarian homografts were grafted beforehand from their intended donors with homografts of skin, then the ovarian homografts were much less frequently successful. With very rare exceptions the hosts that rejected their skin homografts quickly were found to be refractory to ovarian homografts

The experiments described above were carried out it will be noticed, on oöphorectomized recipients. It is the general rule to transplant homografts of an endocrine gland into an animal whose own corresponding glands have been removed—partly because there could otherwise be no functional criterion of the graft's *survival*, and partly because action of the endogenous tropic hormone (which may now be produced in increased amounts and which will not now expend itself on competing tissue) is believed on rather slender evidence to promote the regeneration of the graft. Regeneration is the proper word, for all but the cortical zone of a subcutaneous implant tends to undergo an ischemic necrosis and the graft that may finally establish itself is a regenerate of its surviving cortical cells. Harris and Eakin believe that this chronic tropic stimulation helps the graft to override a certain amount of immunologic opposition as homografts of rapidly growing tumor are quite certainly known to do. But this can hardly be the whole explanation, for a tumor homograft grows progressively whereas a growing endocrine homograft must eventually reach a steady size and state of output.

*The nerve homograft.* The work of Sanders and Young and of Weiss has shown that homografts of peripheral nerve segments are sometimes clinically successful in small animals like rabbits and rats in the sense that their use may lead to at least a partial restoration of muscular function. In man the results of using nerve homografts have been thoroughly disappointing. We need not infer from this that men and rats react against their homografts in constitutionally different ways *scale* comes into it as well The functional success of a peripheral nerve homograft depends upon the degree to which native nerve fibers growing from the central end of a divided nerve can be maintained in working order over the grafted stretch. Sanders has pointed out that the chances of success will be greater if the regenerating fibers can get across to the peripheral stump before the graft is embroiled in the inflammatory accompaniments of the homograft reaction. The chances are therefore greater with short than with long grafts and it is a matter of absolute not merely of relative size For the rate of regeneration of nerve fibers is much the same in rabbits rats and men and so too is the tempo of the homograft reaction. It follows that if the graft is short as it usually

will be in small animals, native nerve fibers may traverse it before its disruption by inflammatory processes. If it is long (as in man it always will be if a homograft is to be used at all) the fibers may well be caught half way through. Thus there is no need to appeal to constitutional differences of reactivity. It is mainly a matter of scale.

## MODIFICATION OF THE HOMOGRAFT REACTION

It is a truism to say that the reaction against living homografts may be modified in alternative ways by changing the antigenic propensities of the grafted cells or the immunological competence of the host. Only the latter will be considered here because one cannot envisage any process of 'de individualizing' a graft before its transplantation that would not at the same time destroy its constituent cells. There is evidence, to be sure, that homografts of tumor cells may be to some degree 'de individualized' in transit through alien hosts, but that is a different matter. Despeciation of such a kind may be due either to the concerted adaptive responses of its individual cells or, more likely, to the selection of the more compatible cells from what cannot be an entirely homogeneous population, and it may take the form either of a true irreversible loss of particular antigens, or of a suppression of antigens, which go into hiding as they do when, as Dr Sonneborn found, *Paramecia* are cultivated in specific antisera. The problem is of the highest importance for the science of somatic cellular genetics, but its clinical bearing upon homografts of normal tissues is still remote.

Some believe that starved or sickly animals are less able, others, that they are better able to resist the transplantation of foreign cells than normal animals, but the contradiction is more apparent than real. The immunologic infirmity produced by various kinds of stress may be more than counterbalanced by the ill-effects of deprivation on the graft as such. The administration of cortisone will beyond doubt lower the resistance of a mouse to the growth of a foreign tumor of lymphoid character, but may yet protect the mouse against such a growth by a direct inhibitory action on the transplanted cells.

There is a wealth of literature on the lowering of resistance to homografts by the x irradiation of their hosts or the administration of colloid matter or trypan blue; the evidence has been ably reviewed by Toolan (25) and requires no further comment here.

### Steroid Hormones and Homograft Reaction

It was shown in 1951 by Billingham, Krohn and Medawar and independently by Morgan that the administration of cortisone to rabbits prolongs the life of skin homografts in rabbits by a factor of three or four; the results of their investigations, together with the more recent work by Krohn and by Medawar and Sparrow (20), may now be briefly summarized.

The power to depress the immunologic response to tissue homografts is sharply confined, among steroid hormones, to cortisol (compound F) and its near relation cortisone (compound E), each is effective as ester or as free alcohol. Progesterone, testosterone, estradiol and desoxycorticosterone (DOC) are ineffective, though corticosterone, in so far as it influences the reaction at all, does so more in the manner of cortisone than in that of DOC. But animals differ profoundly in the sensitivity of their responses. Rabbits and mice are relatively strong reactors to cortisone or compound F in the sense that daily dosages of 5 to 10 mg. per kg. produce a conspicuous prolongation of survival; with guinea pigs a daily dosage of 50 mg. per kg. is needed to produce an effect of comparable magnitude, and monkeys and man seem both to be indifferent reactors. The effects of cortisone and of ACTH do not by any means go hand in hand. Homografts in rabbits are virtually uninfluenced by the administration of ACTH; in guinea pigs, as Sparrow has shown, ACTH is effective in spite of their insensitivity to cortisone, and mice respond moderately well to both.

The cause of these differences is not yet understood, though Krohn has clearly indicated the lines along which an interpretation is likely to be forthcoming. In the first place, the several species differ very widely in the proportions and probably the quantities in which the principal active hormones (viz. compound F, corticosterone and a mineral regulating hormone which is not DOC but may be aldosterone) are secreted by the adrenal cortex. They may also be expected to differ in the rate of utilization of these hormones by the general body tissues and in the sensitivities of the target organs. Until these endocrinologic parameters have been established, as they are now beginning to be, it will not be possible to predict the character of the resp

to cortisone and ACTH on *a priori* grounds. But it is at least intelligible that ACTH should be ineffective in a species in which the principal constituent of the adrenal affluent is a hormone such as corticosterone, which does not prolong the life of homografts or that relatively small extra doses of cortisone will have little effect in an animal in which a high endogenous output of compound F is associated with a low degree of tissue sensitivity (It may be added that, so far as transplantation immunity is concerned the target organ of ACTH is the adrenal gland not even a heavily luteinized ovary can take its place.)

In rabbits (the matter has not been investigated in other laboratory animals) the *local* administration of cortisone acetate in doses as low as 5 mg every third day will at least double the normal survival time of homografts This might tempt one to believe that cortisone acted by some interference at a peripheral level i.e not by preventing the development of transplantation immunity but by denying it a chance to take effect. Cortisone does indeed delay the onset of the inflammatory processes that accompany the destruction of homografts and keeps the grafts in a somewhat indolent or vegetative state. But analysis has shown that the purely local or peripheral effect of cortisone, though it surely exists is trivially small. The systemic or local administration of cortisone does not prolong the life of homografts in rabbits which have already been immunized nor does the local application of cortisone protect homografts against the concomitant immunity produced by untreated homografts transplanted elsewhere on the body It follows then as Krohn has put it that cortisone acts on the afferent side of the immunologic reflex arc Some part of its effect is presumably due to a general and unspecific slowing down of the process of healing and vascularization, and therefore of the process by which antigenic matter gains access to the regional nodes but there seems little reason to doubt that its main effect is to suppress the activity of the cells responsible for the central immunologic response It has been quite firmly established that the regional lymph nodes are the principal anatomic seat of the central reaction and no less firmly established that the administration of cortisone and compound F is responsible for a generalized lymphoid hypoplasia The relationship between endocrine and immunologic activity is therefore probably of a rather loose-knit kind the adrenal hormones controlling the proliferative power and general metabolic activity of lymphoid tissues and so indirectly the efficacy of immunologic response.

Skin grafts on cortisone treated animals are far from normal. Vascularization and strength of healing are delayed as strikingly as in adrenalectomized animals they are both enhanced for a week or more the epithelium remains in a state of mitotic indolence and the general differentiation of the graft is much delayed. Even so the use of cortisone to prolong the life of skin homografts in human beings could be of limited clinical value it would enable the surgeon to conduct a definitive grafting operation at a time chosen by himself in his patient's interests instead of at a time dictated mainly by the exigencies of the immunologic response. Unfortunately the evidence, so far as it goes tends to suggest that human beings are 'bad reactors to cortisone and that the life of homografts could be prolonged to a worthwhile degree only by the administration of entirely unacceptable dosages. Local administration remains the only possibility particularly of the free alcohol of compound F but Woodruff's trials of such a procedure give little ground for optimism. In the present writer's opinion the effect of locally administered cortisol or cortisone on skin homografts in human beings still deserves a thorough investigation though it will be necessary to adjust our minds to the fact that a treatment which cannot work miracles may yet do a certain limited amount of good

### Phenomenon of Enhancement

It is all but universally agreed that tumor homografts and grafts of normal tissues are subject to the same system of laws of transplantation But there is this difference the tumor homograft can sometimes flout these laws and get away with it. Flout is the right word for a rapidly growing tumor may increase to such a size as to kill an alien host not because opposition is lacking but because the opposition that exists has been overcome. It follows that the genetic rules of tumor transplantation are usually simpler than those which pertain to normal tissues. The difference is one of degree Only a limited number of genes (in mice notably those of Snell's $H_2$ series) govern the formation of antigens strong enough to elicit an immunity which will overcome the tumor's energy of

growth Minor antigens may reveal their presence by causing some inflammatory embarrassment and some temporary impediment to free proliferation, but they do not suffice to cause an established tumor to retreat. With skin homografts the distinction between minor and major antigens is somewhat less dramatic for a state of immunity so feeble as merely to retard the free growth of a tumor homograft may destroy a skin homograft altogether

When a transplanted mouse tumor contains one or more major antigens lacking in its host, the general rule is for it to grow for a short while to a palpable size and then to regress. If the host is treated beforehand in such a way as to impair its powers of resistance, the tumor may grow to such a size as to kill the mouse, or—what comes to the same thing—may acquire such a momentum as to break down resistance if any should arise when the effect of the treatment wears away An assemblage of such tests therefore provides an all-or-nothing scoring system which may be used to weigh up the efficacy of treatments designed to subdue the powers of resistance of the hosts. A score of 0 per cent of progressive growths represents the normal state of affairs when the hosts are fully resistant and a score of 100 per cent represents the most complete impairment of response which the scoring system is competent to reveal. Intermediate percentages represent intermediate degrees of interference. In theory such a system of scoring is much inferior to one which records the individual survival times of homografts on individual animals but in practice it makes for quick and informative assays

The system of treatment to be considered here is that particularly associated with the names of Drs. Snell and Kaliss. It has been known for some fifty years that if the prospective recipients of tumor homografts are treated beforehand with various devitalized tissue preparations or extracts therefrom the tumor which would otherwise have first grown and then retreated may grow progressively until it kills its host The systematic study of this phenomenon is the work of comparatively recent years. The reader may be referred to reviews by Snell and by Kaliss (1 27) for a survey of recent work and a tentative appraisal of its significance

The essential facts are these. The tissue preparation commonly prepared by drying from the frozen state must be used *as if* it were intended to provoke immunity It must be injected before the tumor homograft is transplanted (or, at least, before the homograft makes a physiologic connection with its host) a course of injections is generally preferable to a single injection, and the effect of the treatment lasts for the same order of time as an actively acquired immunity *i.e.*, for many months. The treatment is immunologically specific a property first revealed by the superiority of donor or donor strain tissue preparations over any others (and the ineffectiveness of preparations made from the tissues of members of other species) but now most elegantly confirmed by Snell's use of 'isogenic' strains *i.e.* strains differing from each other so far as possible only in respect of single chosen genes. Indeed with tumor homografts the power of a tissue preparation to produce "enhancement goes hand in hand with the possession by its donor of those major genes (notably of the H 2 series) which in living grafts determine their antigenic power

'Enhancement' then is due to an active immunization indeed Kaliss has given excellent reasons for supposing that there is a causal connection between the presence of a high titer of circulating antibodies and the survival of a homograft beyond its normal span of life The paradox is merely apparent. The tissue preparations used to procure enhancement have no power to elicit transplantation immunity what they do (as Gorer first showed in 1947 and many others have since confirmed) is to elicit the formation of serum isoantibodies—antibodies which according to the reasoning of an earlier section are not the instruments that put the homograft reaction into effect. The exact nature of the causal connection between antibodies and enhancement is far from certain one possibility (28) among several is that enhancement is fundamentally a process of desensitization.

What is the clinical promise of the phenomenon of enhancement? In principle very great for here is a treatment which (unlike those which lead to acquired tolerance, in the sense in which that term is used below) can be applied to adult animals and it may at once be said that the phenomenon is by no means confined to homografts of tumors. Unfortunately the use of a scoring system based upon the ability of tumors to kill their hosts gives an exaggerated estimate of the efficiency of the process of enhancement Enhancement appears to give a tumor homo-

graft a period of grace during which it can grow to such a size and build up such a momentum of growth as to overcome the resistance which ultimately develops. Skin homografts transplanted to mice or rabbits which have been prepared by injections of frozen-dried tissues of suitable origin will at best live only two or three times longer than would otherwise have been the case (28) During this period the skin grafts behave normally and may even begin to grow a new pelt of hair Immunity is therefore in abeyance quite long enough to allow the homograft of a rapidly growing tumor to get completely out of control. In other words, a score of 100 per cent in the notation described above can be earned by a comparatively minor and short-lived remission of the immune response.

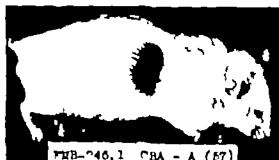



FIG 46 "Tolerance in mice a skin homograft from a CBA (brown) mouse 57 days after transplantation to an albino mouse of strain A The normal survival time is about 10 days The white mouse had been injected while still *in utero* with a suspension of living splenic and other cells prepared from an adult donor of strain CBA



FIG 47 Tolerance in birds a skin homograft from a Rhode Island Red (RIR) donor 282 days after transplantation to a White Leghorn (WL) host The normal survival time of such a homograft is about 8 days Tolerance was achieved by *bringing about a vascular anastomosis (synchorial parabiosis)* between donor and host from the tenth day of embryonic life until hatching skin homografts were exchanged a week after hatching

The feebleness of the phenomenon of enhancement, when measured by the longer and more finely calibrated yardstick of a normal homograft s survival time, must not be allowed to prejudice our estimate of its future promise Billingham and Sparrow (29) have shown that the intravenous injection of a few million dissociated epidermal cells can greatly attenuate the reaction against skin homografts in rabbits This phenomenon may be totally different from enhancement and there is certainly something odd about the properties of the intravenous route in rabbits that is not shared by guinea pigs or mice but it would be foolhardy to suppose that no immunologically specific treatment of an adult animal could ever serve to do away with the homograft reaction altogether The hamster (23) can be called as witness to the truth that the homograft reaction need not happen in the peremptory and brutal form to which rabbits and mice have long accustomed us. It is profoundly encouraging, too that drug allergies"—hypersensitivities produced by application to the skin of drugs such as picryl chloride and dinitrochlorobenzene—can be abolished or greatly enfeebled by so simple a means as the prior oral administration of the drug Drug allergy and the tuberculin reaction it will be suggested, have much in common with immunity to homologous grafted cells.

## Actively Acquired Tolerance

Few generalizations in the field of transplantation immunity are more firmly established than this that the effect of exposing an animal to living foreign homologous cells is to increase the severity of its reaction against cells of the same origin transplanted on some latter occasion. Yet this generalization as a generalization is false indeed there are circumstances in which it expresses the very opposite of the truth. For if an embryonic or fetal mammal or bird is exposed to the influence of living foreign homologous cells, the effect of this prior treatment is to diminish, not to increase, its power to react against foreign cells transplanted later in life and in the extreme case its power of resistance may be done away with altogether so that a homograft of living cells is accepted just as if it were an autograft, and the host becomes a patchwork or chimera of genetically dissimilar cells.

These are the empirical findings upon which the conception of tolerance is founded. A fetal

mammal or bird is by no means immunologically passive although at so early a stage of development it has no power to respond to an antigenic stimulus by immunity, it nevertheless does respond, and in exactly the contrary sense, i.e., by entering a state in which the natural power to react against an antigenic stimulus in later life is permanently and perhaps completely impaired

The study of tolerance may be said to have begun with Owen's discovery that dizygotic twin cattle contain a mixture of each other's red blood corpuscles, the mixing having taken place *in utero* by reason of the fusion of their fetal membranes. Such a mixture might be merely transient in fact, it may be virtually permanent and this must mean that the cells exchanged *in utero* consisted not only of red cells but also of their progenitors, and that in spite of their being homografts these progenitors (or the lineage of cells arising from them) survived. We ourselves later found that dizygotic twin cattle, even when they were of unlike sex, would accept homografts of each other's skin and there can be no doubt at all that mutual tolerance of skin grafts would also prevail in that very small percentage of sheep or human twins which have been found, or will in future be found to be red cell chimeras of a similar kind

My colleagues Drs. Billingham and Brent and I have now reproduced these earlier findings in laboratory animals by experimental means. The details of our methods may be found elsewhere (11) the principle underlying them is simply this. The chosen subject is injected before birth* with blood or more effectively with suspensions of living tissue cells† from a donor of their own species. At some appropriate time after birth or hatching (in mice, six weeks in chickens, two) the subject is challenged by a skin graft transplanted from the original donor or when highly inbred animals are available, from some other member of the donor's strain. The time after birth at which this test is carried out is determined not merely by matters of technical convenience It must be a time when the immunologic reactivity of a normal untreated animal is known to be mature Knowing beforehand the expectation of life of a homograft transplanted from a normal donor to a normal host, one of three results may be looked for The test graft may so to speak fail to live up to expectation in which case the prior treatment could be held to have caused immunity or enhanced resistance it may live out its normal life span and no more, in which case the net effect of the prior treatment would clearly have been nil or it may survive for any length of time beyond its normal expectation of life. It is the third of these possibilities that is normally realized the actual degree of prolongation of survival depends partly on the age of embryonic or immediately post natal life at which the subject was first injected partly on the nature and dosage of the injected cells and their route of administration, and partly on the genetic relationship between the donor and the host. In the more favorable examples tolerance is permanent.‡

Had all these procedures been carried out in *precisely* the same way and with *precisely* the same agents and subjects, except in deferring the first inoculation until a few days after birth then the opposite result would have been obtained—*i.e.* the skin graft used to challenge the injected subject would have lived for less than its normal expectation and the subject itself would thus be held to have been already sensitive or immune. It is not supposed that this strange inversion—from response by negative

* The time at which it is appropriate to inject antigens with the object of inducing tolerance depends upon species strain and route of injection and also probably upon the immunologic system under investigation Billingham and Brent (8) find that the *intravenous* injection of CBA splenic cells into four-day old A line mice can still produce tolerance of CBA skin homografts but injections deferred until the seventh day after birth begin to produce heightened resistance A neutral period lies between

† Difficulties arise over the choice of cell Adult lymphoid cell (including blood lymphocytes) are the easiest to procure and use and are highly effective in producing tolerance *Unfortunately* they will produce overt or subclinical "runt disease (see above Reaction of Graft against Host) Runt disease may be avoided by using foetal blood or lymphoid cells and in mice at least (8) reduced in severity by using adult bone marrow The use of hybrid tissues which are genetically incapable of causing a graft against host reaction is confined to experimental work and has no clinical application

‡ The state of tolerance in the human chimera Mrs McK (9) as revealed by the continued presence in her blood stream *of foreign* red blood cells of group $A_1$ has already been in force for thirty years

immunity or tolerance to response by positive immunity or sensitivity—takes place like a thunderclap at the epoch of birth the quantitative evidence shows that the power of an inoculum to confer tolerance falls away to zero in an orderly manner as the animal grows older and its power to confer immunity starting at zero rises to the *mature value as later development proceeds* but there must be a zero however momentary just as a ball thrown upwards must be stationary for a moment before it begins to fall and this zero is revealed by the second of the three possibilities listed above There is a short period of a mouse s life at which the inoculation of foreign cells confers neither tolerance nor immunity the test graft enjoys its normal lifetime, neither more nor less.*

Something should now be said of the immunologic properties of the state of acquired tolerance It is immunologically specific A mouse made tolerant of grafts from one donor will reject grafts from a second donor—unless, in deed the two donors should happen to be members of the same inbred strain and of the same sex in which case they count genetically and therefore antigenically as one. A tolerant mouse *ceases to be so* and becomes sensitive or immune if it is injected intraperitoneally with cells expressed from the regional lymph nodes of an actively immunized mouse of its own strain The donor of the lymph node cells must belong to *the same strain as the tolerant mouse for otherwise* the transferred cells would themselves behave as homografts and would be destroyed by the tolerant mouse before they had time to exercise their effect.

By this means a tolerant mouse which has been carrying a homograft for a hundred days or more may be restored to a state of normal reactivity within ten days or less the homograft tolerated until then withers away and is sloughed off as a dried up scab It is a matter of perhaps deep theoretical importance that the same effect can be achieved though somewhat more slowly (in 20 to 40 days) by injecting the tolerant mouse with *normal* lymph node cells from unimmunized mice of its own strain What happens we suppose is that these normal lymph node cells become incorporated in the tolerant mouse and thereupon react against the antigenic matter liberated by the tolerated homograft the tolerant mouse becomes normally reactive by being surgically equipped with immunologically competent cells. This *experiment shows that the tolerated* homograft continues to be a source of antigenic stimuli—or rather of stimuli which would be antigenic if the host were capable of responding to them and from it one may infer that normal *tissue cells are constantly shedding subcellular* fragments or particles which as part of some normal mechanism of tissue clearance are wafted through the lymphatics into the cells of the regional nodes. In normal or in tolerant mice these particles, being autogenous or effectively so provoke no immunologic response They exercise their antigenic power only when they gain access to lymph node cells to which they are foreign This as we have seen may be brought about by alternative means (*a*) in normal mice by transplanting tissues between members of two different strains (*b*) in tolerant mice, by causing normal lymph node cells to enter what would otherwise be inert or immunologically incompetent nodes. In the one case normal homografting, the graft is given access to reactive nodes in the other reactive nodes are given access to an otherwise tolerated graft. Whether Mahomet goes to the mountain or vice versa is relatively unimportant. But it is a most remarkable fact that complex matter some of it perhaps of nuclear origin, is constantly liberated by normal tissue cells and it is not easy to see how any *other type of experiment could reveal the phenomenon* so clearly What the *normal* function of this system of clearance may be is at present conjectural. It may be no more than merely excretory but it could conceivably be an ingredient of some *deep seated process of biologic* control

So much for what is purely speculative Taken at their face value, these experiments on the restoration of normal reactivity to tolerant mice show that tolerance is due to a *central failure* of the mechanism of immunologic response and not to an interference with either the afferent or efferent side of the immunologic reflex mechanism. The tolerated graft continues to manufacture would be antigenic matter and it continues to be able to respond to an immune state induced by proxy the tolerant mouse does nothing to deny such an immune stat it power to take effect These were the *first objects of our* experiment and these at least have been achieved

* This is the neutral period referred to in the footnote on the preceding page

Homografts and the modes of reaction associated with them are particularly well suited to the demonstration of tolerance, because living cells provide a continuing or chronic antigenic stimulus. To achieve an effect of comparable magnitude with the ordinary non-living antigens of classical immunology, a single fetal inoculation will often not suffice. But there are already clear indications that the phenomenon of tolerance is not confined to "iso-immunity" reactions nor to antigens attached to cells.* The predictions of Burnet and Fenner have thus been handsomely fulfilled: for knowing only of Owen's work beforehand they predicted results of just the kind which we ourselves have since obtained.

The phenomenon of tolerance has a certain important bearing on the theory of antibody formation. The current theory, in a nutshell, may be put thus: antibody formation is a specific diversion or redirection of protein synthesis brought about by its performance under some kind of molecular constraint. If this were the whole story, then embryos, in which protein synthesis is particularly vigorous, should be adept at making antibodies, and it has always been something of an embarrassment to immunologically minded biochemists that they are not. Our evidence shows that embryonic cells are very far from being non-reactors to antigenic stimuli: they do react, but it just so happens that their reaction is, by adult standards, in a negative sense. Nor need the fact that antibody formation is apparently confined to certain specialized cells and not shared among all those that indulge in protein synthesis, be regarded as an obstacle to the accepted theory. Consider, for example, nervous conduction and muscular contraction. The propagation of that change of state which, when it occurs in nerve fibers, is called an impulse, is by no means confined to nerves. Something of the kind almost certainly occurs over most cellular surfaces, though the cell must be long and thin (*cf. Nitella*) if the change is to be easy to detect. Many cells other than muscle fibers contract or contain contractile elements; probably every cell has some rudimentary power to shorten its fibrous molecules reversibly. In short, what distinguishes neurons and muscle fibers is not that they propagate impulses or have power to contract, but that they perform these functions superlatively well. So it may be with antibody formation. Probably, as biological chemists have suggested, antibody formation or something akin to it is an "elementary" property of systems indulging in protein synthesis; the cells of lymph nodes and of the spleen are distinguished only by having this property developed to a particularly high degree.

One further implication of the phenomenon of tolerance deserves to be considered: it bearing upon the isolation of embryos from the outside world and particularly the shutting off of the mammalian fetus from even the finest open connection with the blood stream of its mother. On purely physiologic or at all events hydrodynamic grounds, there seems no good reason for so very strict an isolation. Immunologically it is intelligible for two equally valid but exactly converse reasons. That the mother should be denied access to antigenic matter arising from the fetus is obvious enough when we consider the evil consequences of maternal immunization on the rare occasions on which it does occur. It is probably of equal importance that the fetus should be protected against the ingress of antigenic matter of any kind from the mother, not so much because the fetus would have no protection against it (maternal antibodies or naturally occurring antibacterial substances should look after that), as because the fetus's power to develop resistance on its own account after birth might be seriously impaired.

Tolerance is not itself a phenomenon that promises anything of immediate clinical value in the surgery of tissue repair,† but ma much

* Evidence from many sources, too numerous to be cited individually here, shows that tolerance can be produced by purified soluble heterologous protein, purified enzymes (including enzymes of microbial origin), mucoid substances of the type responsible for bloodgroup specificity, viral and other microbial antigens, organ-specific tissue antigens, and so on. It is becoming increasingly clear that a brief acute exposure to antigenic stimuli is *not* sufficient to produce a lasting tolerance. My colleagues and I have not yet been able to produce tolerance of homografts with anything but living cells, and are perhaps unlikely to succeed until highly concentrated antigenic preparations become available.

† This judgment is based upon two considerations: (a) that it is hardly feasible to attempt to confer tolerance on human beings by cellular injections made before birth, and to delay it until after birth may be to delay too long; and (b) that unless some kind of cellular cocktail is injected

as even the purest and most abstract scholarly enterprises may be tainted by some measure of practical usefulness the following deduction should be kept in mind. Tolerance of skin homografts may be induced by the inoculation of fetuses with blood. The antigenically active ingredient of blood is the white blood corpuscle. It follows then, according to the reasoning set forth in an earlier paragraph that the skin contains no "transplantation" antigen that is not present in the white corpuscle. If it did the leukocytes would not confer tolerance of skin skin would contain a residue of antigens not shared with the leukocyte and there would of necessity provoke an immune response. If therefore it ever turns out to be possible to subdue transplantation *immunity to a worth while degree by injecting* the recipient of a skin or kidney homograft with modified antigenic matter from his intended donor then these modified antigens need not be prepared from skin or kidney as the case may be They may be prepared from blood or the leukocytes of blood, *i.e.*, from the tissue which is easiest to come by and from the donor's point of view the easiest to spare

## NATURE OF TRANSPLANTATION IMMUNITY

Until the elucidation of the causes of hemolytic disease of the newborn and of the nature of allergy and atopy it was possible for a charitably minded pathologist to believe that all immunologic reactions were beneficial, even when the nature of *the benefaction was far from clear More often* than not he was probably right for example, most pathologists assumed on principle that inflammation performed some highly useful protective function, despite appearances to the contrary and the power of anti-inflammatory drugs to allow the systemic spread of what would otherwise have been a localized infection has now entirely borne them out. For all that some immunological reactions do act anti-adaptively *i.e* to the detriment of their subjects and the failure of homografts like hemolytic disease is a teleological misadventure of just this kind. It is true that the immunologic nature of the homograft reaction is still, in some circles, thought to be controversial, but this view in the present writer's opinion, is pure flat-earthism. Indeed, the trouble with describing the homograft reaction as "immunologic" is not that it is too particular a description, but that it is much too vague. Instead of fretting about whether or not the immunologic interpretation is true, students of the problem would be better occupied in trying to find what kind of an immunologic reaction it is. Of what kind is it?

Seen in broad outline, the homograft reaction conforms to a classical immunologic pattern. *Resistance to homografts is actively created*, not inborn or ready made. The speed with which resistance builds itself up to an effective level depends upon the degree of foreignness of the graft the amount of the tissue that is grafted, and also presumably on the time it takes the antigenic matter issuing from the graft to reach the centers of response. In rejecting a homograft, its recipient enters a refractory or specially resistant state, so that a later homograft is reacted upon more violently and more quickly than its predecessor The refractory state is not confined to nor even specially effective within that part of the body in which the original homograft was destroyed it extends to all parts of the body accessible to blood vessels or blood borne cells. By and large we should not go far wrong in thinking of a homograft as the cause of a disease of which its rejection is the cure.

But the more closely the homograft reaction is examined, the more widely it is seen to depart from the "classical pattern—*e.g* in the very long duration of the sensitivity that is caused by the transplantation of a 'solid homograft, and in the fact (if it is a fact) that circulating antibodies do not, or at least need not take any part in the reaction by which homografts are destroyed

The homograft reaction could be a reaction *sui generis* the sole occupant of a category all its own It enjoys however a combination of properties that ally it unmistakeably to the tuberculin reaction and to hypersensitivity reactions of the delayed type * It is a reaction mediated not by

any *state of tolerance that can be produced in* human beings will be confined to grafts from one donor *viz* the donor who provided the cells that were injected before or shortly after birth Nevertheless certain recent observations by L A Peer and by M F A Woodruff are moderately encouraging in so far as they suggest that an injection deferred until after birth may not be entirely inefficacious

* The analogy between transplantation immunity and the tuberculin reaction was first proposed

serum antibodies but by immunologically activated cells, the methods by which Mitchison and, following him, we have been able to secure the 'adoptive transfer' of transplantation immunity are with minor exceptions formally analogous to the transfer of drug allergy and tuberculin hypersensitivity The tempo pattern, and systemic compass of transplantation immunity give the analogy extra force Transplantation immunity like the hypersensitivities is vulnerable to the action of adrenal steroids of the cortisone family but is apparently untouched by antihistaminic drugs. Transplantation immunity and hypersensitivity are alike in being abolished by extensive x-irradiation—in both cases, presumably because of some deep-seated injury to the lymphoid system Moreover immunologic responses of both kinds fulfil themselves in a violent and, in extreme forms, necrotizing inflammation over which the round cell rather than the polymorph presides. Finally Brent, Brown and I (36) have just shown that transplantation immunity in guinea pigs can be made to manifest itself as dermal hypersensitivity of the delayed type If "homograft antigen extracted from the tissues of the donor is injected intradermally into a guinea pig made sensitive by the transplantation of skin homografts a delayed tuberculinlike inflammation develops at the point of injection. The reaction is transferable by sensitized lymphoid cells but not, apparently by 'immune serum The transfer reaction takes the form of injecting regional lymph node cells from the sensitized recipient into the skin of the homograft donor a position in which needless to say homograft antigen" is already present

These affinities can hardly be fortuitous. They suggest that reactions of both kinds proceed for some length at least, along a common causal pathway Perhaps everything that the two have in common can be summed up by saying that both depend upon the activation deployment and peripheral engagement of the lymphoid cell When we add to this statement the rider that we are almost completely ignorant of the normal function and fate of lymphocytes our inability to be more specific can hardly cause surprise

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by F M Burnet and F Fenner and it has been developed by several others since e.g Mitchison (18) Favour (30) and Medawar (22)

## SURGICAL IMPORTANCE OF THE HOMOGRAFT PROBLEM

The 'homograft problem' owes its surgical importance not to the number but to the gravity of the occasions on which homografts might be used. Homografts will never be needed very often, but, when they are needed at all, they are needed with extreme and sometimes mortal urgency Is then the homograft problem soluble in a surgically effective sense? Will there come a time in which tissues and organs can be transplanted at will between different individuals so that surgery can enter that 'new dimension of accomplishment for which it has had to wait so long? All things considered it seems reasonably likely The phenomenon of tolerance shows that the clinical problem is soluble in principle, and the existence of a phenomenon of 'enhancement' in its various forms shows that the immunologic defences of even adult animals are by no means so impregnable as we were formerly inclined to believe Unfortunately, the reaction of the host against a homograft is not the whole story we need now most urgently to know if the reaction of the graft against the host will make kidney lung and other complex homografts unusable even when the host can be induced to accept them There is nothing in the history of transplantation to suggest that these problems will yield to anything but informed and careful experimentation—an activity which can be entrusted neither to the laboratory recluse nor to the practicing surgeon but only to a judicious cooperation between the two


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# PART II

# *Skin*

CHAPTER 3

# *Transplantation of Skin*

BLAIR O ROGERS

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Wilhelmina M was a 33-year-old unmarried woman, at one time considered an absolute beauty who lived in retirement in the country because her nose adjacent cheek and eyebrow regions had been destroyed by a chronic skin eruption of 15 years duration Herr Professor Dr Christian Bünger of Marburg in 1823 described his pioneering attempts to reconstruct a nose for this pitiful lady by transplanting a free full thickness skin graft taken from her thigh (1)

Following the *Ancient Indian Method* of rhinoplasty (2) in which skin grafts were presumably taken from the buttocks he relates

> I pre-selected skin from the thigh and according to accepted practice In order to increase its vitality I slapped it long enough until a moderate degree of redness and turgidity had taken place in it
>
> I determined the size of the piece of skin required by the superficial use of a paper measure and for a fast practical closure of the thigh wound I removed more skin than was necessary in the form of an oval roughly 4 inches long by 3 inches wide
>
> I let the separated piece of skin lie in my left palm where I fashioned the required shape by cutting away excess tissue and it was during this procedure that I found I had to remove a good deal of fatty tissue which was more than was needed in the middle portion of the graft I did this in order to make possible a truly first rate exact union of graft edges with the edges of the facial skin defect

One can easily share with Bünger his early enthusiasm and excitement when he observed the successful "take" of this historic graft which at the time of its operative removal from the thigh had appeared precariously or deadly pale and had "apparently lost its own warmth" prior to its application to the nasal defect. Bünger exclaims describing his follow-up visits to the patient on the third postoperative day

> I had strongly wished that on this morning more members of the department could have had the opportunity that was ours to observe what we saw when we opened the dressing!
>
> All that we doctors could do was merely to stare and at first we could not trust our own eyes for we saw that the graft which had only a day before been deathly pale and which at the very least had been separated from the body for an hour and a half during the operation was now still holding its shape as a nose with a considerable part of its surface having a shiny turgid scarlet red color

When five weeks had passed and a fairly well shaped nose was the result of this daring free skin transplantation Bünger concluded

> All in all it was successful and from a physiologic point of view it was truly remarkable for even if a nose had not been formed it was a good demonstration that a fully-separated skin graft that had been detached from the body itself for more than an hour still maintained all the vital requirements necessary for its re attachment to the parent skin

Bünger's fascinating account is probably one of the earliest, if not the first fully documented report by a physician of free autografting of skin to appear in the medical literature. It serves as a good example moreover of one of the two basic methods of skin transplantation

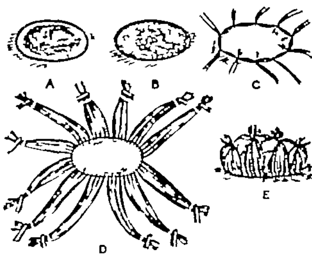



FIG 48 One method of repairing a skin defect with a free skin graft using interrupted sutures and leaving long ends which are tied over a bolus dressing A The incision about the lesion B The defect C The skin graft cut to pattern being sutured in place D Final suturing with suture ends gathered in groups of four E Suture ends tied over a gauze bolus (From J P Webster Plastic Surgery General Principles in Textbook of Surgery ed by F Christopher p 1403 W B Saunders Company Philadelphia 1940 )

One of these *free* skin transplantation or *free* skin grafting (fig 48) implies that the skin is completely detached from the body and thus from its underlying blood supply when it is transferred to another part of the body The word *free* and *graft* therefore should be considered synonymous in any discussion of *methods of skin transplantation*

The other basic method consists of transplanting skin pedicles or flaps. Use of the word *pedicle* or *flap* implies at all times that the skin and its underlying subcutaneous tissue remains attached temporarily or permanently to the body where it continues to receive its blood supply through this pedicle attachment or flap

Since the over-all problems of tissue transplantation are largely concerned with grafts *e g* bone grafts and corneal grafts and not with flaps the discussion of skin transplantation that follows will be confined to the problem of free skin graft The historical clinical, and surgical detail of transplanting skin pedicles or flaps are competently dealt with in many well written book and articles on plastic and reconstructive surgery (3 6)

## Types of Skin Grafts

Three types of skin graft will be discussed in this chapter the *autograft* obtained from one part of the body and transplanted to another part of the selfsame body the *homograft*, obtained from one individual and transplanted to another individual of the same species—*e.g.*, man to man rabbit to rabbit and the *heterograft* obtained from an individual of one species and transplanted to an individual of another species—*e.g* man to guinea pig chicken to rat.

## Function of Skin Grafts

Sir Harold Gillies and Ralph Millard, in their recent fascinating book, *The Principles and Art of Plastic Surgery* (3) sum up the function of a skin graft from the clinician s standpoint as follows

> A skin graft plays three main roles in plastic surgery 1) the leading part where it serves as the final result 2) an assisting part where it serves as cover and disguise of the donor area denuded by flap or Wolfe graft for the primary condition 3) a stand in part in the form of a temporary dressing of the raw area to be discarded later

What role does a skin graft play in basic scientific research in the laboratory? Recent symposia devoted to advances made in the past decade in tissue and organ transplantation have certainly demonstrated that skin grafting has become a very important laboratory tool for diverse specialties such as genetics immunology oncology pathology zoology morphology embryology endocrinology and so on.

The whole science of tissue transplantation in the clinic as well as in the laboratory probably owes its development largely to the recent experimental and clinical research findings in the transplantation of skin (7–15) Within the past decade for example it has almost been impossible to pick up an article on the homotransplantation of any tissue without observing some reference to the pioneer skin grafting experiments of Professor Peter Medawar and his colleagues at the University College London, Department of Zoology (8–10) The implications of this state of affairs are self-evident. Any understanding of the biologic laws of transplantation requires good comprehension of the behavior of skin autografts and homograft and *vice versa* (see chapter 2)

Similarly any understanding of the biologic laws of skin homografting and skin heterografting requires a working knowledge of the standard behavior of skin autografts Obviously skin autograft and homografts are the most

readily accessible tissues for transplantation experiments

## EARLY HISTORY OF SKIN GRAFTING

The ancestry of skin grafting procedures has been traced back to the seventh or eighth centuries in the pre-Christian era (16) when Hindu surgeons of the Tilemaker caste were known to transplant flaps of a patient's forehead skin to repair amputated noses. These same pioneers are also said to have reconstructed the nose with free autografts of skin taken from the buttocks but medical historians find it difficult to trace this latter procedure as far back as the descriptions of flap transplantation described by the Hindu surgeon Sushruta in his original Samhitá, written about 750–800 B C (16–17)

The challenge of successfully homografting skin from one human being to another has been the main problem of transplantation research for the past decade. Its solution has fascinated surgeons for many centuries. Calenzio in 1503 praised the surgical skill of a Sicilian plastic surgeon Branca, who reputedly borrowed skin from a slave to reconstruct his master s missing nose (18) Although experience has shown that this form of homografting undoubtedly failed, it serves as a good example of the antiquity of this surgical procedure.

Sir Harold Gillies writes of the above episode in the following colorful manner (3)

> It was failure at cross-grafting in the sixteenth century that was responsible for plastic surgery s centuries of disrepute There had been successes in autogenous grafting but in the absence of anesthesia the process was most painful In those days when the line between employer and em ployee was more distinct it was natural for a master to permit his slave not only to contribute a bit of skin but also to share the greater part of the pain During the following weeks the united slave and master got to know each other quite well and as soon as their plastic attachment was divided the slave was sold Legend reports that one master s new nose which had been quite satisfactory suddenly grew cold and dropped off Upon investigation it was found that the slave had died in Brussels that same day
>
> "In the seventeenth century Samuel Butler was still jesting about this practice of having a porter slave contribute a bit of his bottom for his master s nose when he wrote
>
> "But when the date of Nock was out
> Off dropt the Sympathetic Snout

In addition to these early attempts at skin autografting and skin homografting—some successful, others more often disastrous—one *of the most bizarre chapters of clinical medicine*, that of skin heterografting must also be mentioned here Reverdin (19) suggested in 1871 that grafts of skin could be taken for human use not only from another human being but from a different species as well. As a result one year later Coze (20) reported the healing of three cases of chronic obstinate leg ulcers with skin grafts supplied by rabbits. "In this way the era of free skin zoografting began (21)

John Staige Davis (2) one of the great pioneers in American plastic surgery, described in his textbook of 1919 his experiences with zoografts. The term *zoograft* itself is a substitute for the word heterograft, being employed whenever the host is a human being and the donor an animal. Davis used skin taken from a wide variety of animals and fowl including rats rabbits, young puppies, guinea pigs pullets and pigeons He was not too pleased with the clinical results of zoografting Although these grafts seemed to have the power " of stimulating epithelial growth from the edges when placed on granulating wounds, as do other grafts, after doing well and often when the wound was entirely healed suddenly and with no apparent cause the grafts routinely melted away and soon disappeared.

### Development of Plastic Surgery and Skin Grafting

The art of reconstructing noses with flaps and grafts of skin apparently was carried from ancient India by way of southern Asian trade routes and the historical currents of Persia and Arabia finally reaching the Mediterranean civilizations particularly those in Sicily and Italy (22) With the advent of a cultural renaissance in Europe the art of transplanting skin by means of flaps was apparently revived from the archives having lain dormant for those centuries known as the Dark Ages It was during this renaissance that the fifteenth century poet, Calenzio referred to Branca of Sicily a forerunner of the Bolognese surgeon and anatomist Gaspare Tagliacozzi. Branca the elder and his son Antonio apparently left no writings to illustrate their methods of reconstructing the nose with flaps of skin taken from the cheek and arm but references to their work by Calenzio

*and other writers of the time coupled with* accounts of the surgical procedures practiced by the Vianeo family in Calabria reached Tagliacozzi, who is generally considered the father of plastic surgery as it is known today (23)

It was at least two centuries later however before the Ancient Indian method of rhinoplasty and the Tagliacozzi method (utilizing double-pedicled skin from the arm region) were again employed successfully in continental Europe (1 22-23) A second-hand report of the possible first use of a free skin autograft was given by Leroux who in 1817 described an operation which in turn had been described in a series of verbal and written communications originating in the armies of the Mahrattan prince Scindiah of Western India (24) In this operation the nose was reconstructed by Hindu surgeons who *slapped the donor site of the free skin graft in* the buttocks region with a slipper to produce redness and tumefaction before removing the skin for transplantation. Bünger (1) in 1823, was probably the first European surgeon to make successful use of this procedure but he chose the thigh instead of the buttocks as a donor site much less subject to postoperative discomfort

Bünger's free graft measured 3 by 4 inches and was oval in shape Applied 1½ hours after removal it was largely successful Other surgical procedures a year later helped to complete the nasal reconstruction in a few small areas where the original graft had not taken completely It is interesting to speculate whether Bünger's success was inadvertently guaranteed by his cutting away excess fatty tissue from the middle portion of the graft thereby permitting a more rapid and easily facilitated nourishment of the full thickness graft by the underlying host-bed vessels

Nineteen years prior to Bünger and thirteen years prior to Leroux Baronio (25) a Milanese physician obtained the same successful results with free full thickness skin autografting in animals In 1804 he conclusively demonstrated this procedure by three sets of experiments performed with a sheep In the first experiment he took two pieces of skin of equal size and shape without any subcutaneous fat, from opposite sides of the base of a sheep's back in the tail region and immediately cross transplanted them to their correspondingly opposite defects Eight days later the dressings over these auto-*grafts were removed and the grafts were observed* to be normally healed in place

In a second experiment he cut two additional full thickness grafts, also without any underlying subcutaneous fat and transplanted these to their corresponding defects on the opposite sides of the same sheep's back but somewhat more cephalad to the first set of grafts. These grafts however were transplanted eighteen minutes after their removal following their inspection by the master of the house in which the animal experiments were performed. These too were normally healed in place when inspected on the eighth postoperative day

A third set of experiments was also performed on this same long-suffering sheep in which two other full thickness grafts including their underlying subcutaneous fat and muscle fibers were *cross transplanted to their corresponding defect* in the shoulder region, several weeks following the two previous experiments. On the eighth postoperative day they both looked moribund and it was necessary to express some pus from their beds On the thirteenth postoperative day one of the grafts appeared normally healed in place whereas subsequent follow-up observations of the opposite graft suggested that it had sloughed

With the report of Bünger one might have expected a widespread application of this new method of free skin autografting Bünger's contemporaries however including Dieffenbach, von Graefe Walther and Wutzer unfortunately were not successful at free skin grafting in the human (10) and this probably had a dampening effect on any enthusiasm for this technique Only a few verified reports of success appeared in the medical literature prior to Reverdin's monumental paper in 1869 (20) Among these were the reports by two Americans Jonathan Mason Warren of Boston (27) and Joseph Pancoast of Philadelphia (28)

Baronio's significant findings went unnoticed little attention was given to Bünger's report and it was not until more than fifty years later that free whole thickness skin grafts came into general use In 1869 J L Reverdin reported the hastening of the healing of granulating wounds by what he called epidermic grafts but which he later admitted contained a portion of the corium (23)

## CLASSIFICATION OF SKIN GRAFTS

Reverdin (26) studying problems of wound healing in ulcers speculated that epithelization

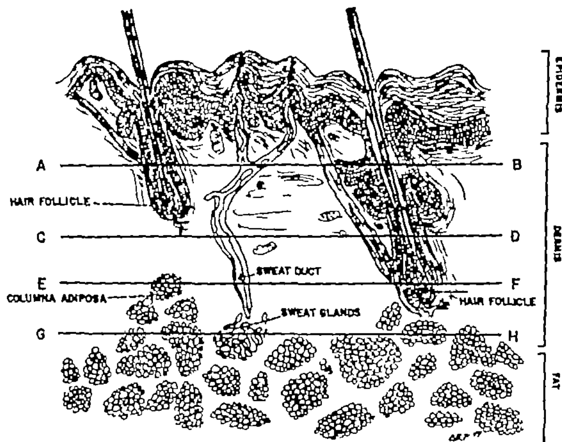



FIG 40 Diagram of skin showing levels at which skin grafts are removed AB Level of an Ollier Thiersch graft CD Level of an intermediate split graft EF Level of a thick split graft GH Level of a full thickness skin graft (From J M Converse and A. H T Robb-Smith The healing of surface cu taneous wounds its analogy with the healing of superficial burns Ann Surg 120:873 1944 )

of a granulating surface could probably be hastened by implanting small islands of epithelium on the granulations In a patient who had lost some of the skin of his thumb Reverdin cut two morsels of skin from the arm each approximately 1 sq mm. and placed them on the granulating thumb defect, immobilizing them with lead plaster strips. It took only a few days for those small grafts to proliferate and grow out to the very margins of the wound defect. Reverdin's report of this case to the Société *Impériale de Chirurgie* on December 8 1869 a report which detailed the more rapid healing of granulating wounds by the use of very small, thin pieces of skin completely detached from their native beds aroused a stimulating discussion by this same Society one week later While neither his paper nor the ensuing discussion was immediately appreciated they served as the spark which was needed to start the flame of interest in the subject of skin grafting throughout the world (16)

Reverdin's little grafts are frequently called *pinch grafts* because the surgeon cuts them by picking up a very thin piece of superficial skin with the point of a needle or pinches such with a forceps held lightly in one hand and with the other hand slices the little cone of skin thus formed away from its base with a sharp razor blade, scalpel or knife.

Following Reverdin's monumental reports (26 29) L. X. E. L. Ollier of Lyon in 1872 described the successful transplantation of much larger films or sheets of epidermis measuring roughly 4 6 or 8 cm square (30) Because these sheets of skin consisted of the entire thickness of epidermis but only a very thin superficial layer of dermis, they have been termed "*thin split thickness grafts*

Although Lawson (31) and Le Fort (32) used full thickness skin grafts (fig 40) in 1870 and 1872 respectively for the repair of ectropion Wolfe (33) of Glasgow is generally given credit for having introduced the whole or "full thickness graft into clinical practice in 1875

In 1886 Carl Thiersch reported to the Fifteenth Congress of the German Surgical Association his perfections in skin grafting which were in essence a use of large sheets or film of thin split thickness skin containing the entire

epidermis and a very thin layer of dermis (34) Thiersch made no mention of Ollier however who had transplanted this same type of thin split thickness skin graft fourteen years previously (Perhaps Thiersch could not or did not read French medical literature!) Medical historians have corrected his oversight, nevertheless and these grafts are known today in some centers as "*Ollier Thiersch grafts*" This cumbersome appellation is more frequently shortened merely to Thiersch grafts however and thus it seems that Herr Dr Thiersch s oversight has survived despite the kind efforts of the historians.

Because Feodor Krause (35) actually popularized the use of full thickness grafts by his perfected methods reported in 1893 medical terminologists in fairness have assigned the title of Wolfe-Krause grafts to full thickness or whole thickness skin grafts. Krause suggested quite wisely and a subsequent half century of plastic surgery has amply demonstrated, that full thickness grafts are usually indicated whenever Ollier Thiersch or thin split thickness grafts have proven unsatisfactory or inadequate

In 1914 John Staige Davis described the superiority of using deeper skin grafts than those obtained by Reverdin s techniques (36) Whereas Reverdin s grafts included only a very thin smidgen of superficial dermis the *small deep grafts* or *Davis grafts*" included at their centers almost the entire full thickness of skin with the deepest layers of dermis serving as an integral part of the graft. However because both the area thus grafted and the donor site when healed have an unsightly pebbled or cobblestone appearance the Davis small deep grafts have largely been replaced by the use of pieces or sheets of *intermediate*" or *thick split thickness skin*

In recent years modified Ollier Thiersch grafts have proven more satisfactory in many surgical unit Contrary to original descriptions they are now cut thick enough to include at least one half of the thickness of the underlying dermi Such a graft has been called a "split thickness intermediate thickness or Blair graft in honor of Vilray P Blair one of the great pioneers of American pla tic surgery who popularized it use (37) Some Blair grafts" if cut even thicker so that they include approximately three quarters of the thickness of the dermis are termed *three quarter thickness grafts* or *thick split thickness grafts*

This brief introduction to the early beginnings and classification of skin grafting does not truly indicate the vast amount of clinical and experimental study devoted especially within the la t half century to autografts and homografts particularly the latter Peer (38) has recently emphasized that even these numerous studies rest uneasily on a rather weak scientific base because relatively little work has been conducted to define clearly the elementary behavior physiology morphology and pathology of that most frequently used type of graft namely the skin autograft, whereas much time comparatively has been expended in all manners and sorts of experiments with skin homografts (11–15)

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## PHYLOGENETICS AND EMBRYOLOGY OF SKIN

Recent research in skin homografting has shown that skin shares some antigens with every other tissue of the body with the possible exception of erythrocytes (39) Since some of these antigens seem to act as foreign-proteins" directly responsible for the rejection of skin homografts by most individuals, biologists are now interested in the exact loci or source of this antigenic material. Are these antigens confined to the epidermal cells dermal cells epidermal mitochondria basal cell centrosomes etc.? Is this ability of skin to share antigen systems with other body tissues and organs explained in part by its common phylogenetic and embryologic interrelationship with other tissue systems derived from embryonic ectoderm?

Any complete study of the behavior of skin autografts and homografts should probably consider these interrelationships. In such consideration one is immediately confronted with the fact that adult skin is formed from two embryologic layers the epidermis being derived from embryonic ectoderm and the dermis from the mesenchymal or mesodermal layer Since the mesoderm is initially formed by cells budding off in the early embryo from the "prim-

itive streak' (the latter itself having originally developed as a keel-like thickening of embryonic ectoderm) adult skin might literally be said to be derived originally and entirely from primitive ectoderm. But this may be pressing a regression too far.

The epidermis is only one of many adult organ and tissue systems derived from this embryonic ectoderm, and the dermis in a like manner is only one of countless other tissues of the body which have mesenchymal or mesodermal origins. Since Gaines (40) has clearly outlined the interrelationship between some disorders of the skin and other body systems based upon their mutual phylogenetic and histogenetic derivation from embryonic ectoderm, one can easily understand some of the mechanisms that lead to a common sharing of antigens by these selfsame systems.

Carrel (41) spoke of the human body as a closed universe which is limited on one surface by the skin and on the other surface by the mucosa. This analogy can be applied equally to the earliest forms of primitive life or to the primitive embryo of most animal life. The primitive vertebrate and most invertebrate embryos consist merely of a simple mesodermal layer limited or covered on one surface by an ectoderm (or "skin") and on the other by an entoderm (or "mucosa") the latter being developed as an invagination of the former.

In simple protozoan cells ectoderm actually serves as a special pathway of sensation (42). The nervous system of jellyfish and sea anemones, for example, is merely a special thickened portion of the ectoderm or outer skin (43). Although the earthworm does not have any special sense organs, it still responds to sounds, odors, light and orientation. Its responses are the result of stimuli reaching modified epithelial cells in its skin which are specifically sensitive and conductive.

Early in evolution portions of ectoderm have been specifically modified to serve as touch receptors (40). Biologists report that ectodermal epithelial cells have also been modified in higher animal forms into organs that are primarily responsible for the sense of light, hearing, and equilibrium. Every past medical student can recall in his training an emphasis placed upon the development of the nervous system with its earliest appearance in the form of a thickening of ectodermal cells known as the neural plate.

The neural plate further develops into the neural groove and thus into the neural tube. Groups of ectodermal cells on both sides of the neural tube, known as neural crests, then extend themselves along the tube and ultimately develop into the posterior spinal root ganglia. From these ganglia cellular prolongations are sent outwardly to the skin's epithelial layers where they are broken up into minute end-organs responsible for the sensations of pain and touch. A neurologic cycle of embryologic development has thus been completed—a cycle originating in ectodermal cells known as the neural plate and terminating in epithelial end-organs also derived from ectoderm.

The interrelationships between skin and the neurologic system just described as a mutual evolutionary and embryologic process are clearly demonstrable in certain disease syndromes seen in human beings, *e.g.* shingles (herpes zoster). Andrews (44) unintentionally describes such an interrelationship in the following account of a clinical case of herpes zoster.

> Herpes zoster is characterized by groups of vesicles on an erythematous base situated along the distribution of nerves from one or more posterior ganglia, the onset being rapid, sometimes with fever and neuralgic pain. It is generally believed that the virus enters by the nose and becomes localized simultaneously in ectodermal structures and tissues of the sensory nervous system. It may travel from the cortex to the periphery, or *vice versa*, inoculating the neural structures along which it travels.

These interrelated skin and neurologic disturbances are duplicated in other disorders that affect the skin and endocrine glands, and the skin and alimentary tract, since these systems also share their phylogenetic and embryologic derivation in most instances. For example, from the same cellular system which splits off from spinal nerve ganglia to form the sympathetic nerves, another type of cells, known as chromaffin cells, are formed which eventually can be traced in development to the adrenal medulla, carotid body and sympathetic glands lying in front of the aorta. A stimulus to the adrenal, such as an acute alarm, will usually result in a general stimulation of endocrine and sympathetic systems alike, resulting in certain specific interrelated skin effects, many of which can be attributed to the release of adrenalin.

epidermis and a very thin layer of dermis (34) Thiersch made no mention of Ollier however who had transplanted this same type of thin split thickness skin graft fourteen years previously (Perhaps Thiersch could not or did not read French medical literature!) Medical historians have corrected his oversight, nevertheless and these grafts are known today in some centers as *Ollier Thiersch grafts* This cumber some appellation is more frequently shortened merely to "Thiersch grafts," however and thus it seems that Herr Dr Thiersch's oversight has survived despite the kind efforts of the historians.

Because Feodor Krause (35) actually popularized the use of full thickness grafts by his perfected methods reported in 1893 medical terminologists in fairness have assigned the title of "Wolfe-Krause grafts" to full thickness or whole thickness skin grafts. Krause suggested quite wisely and a subsequent half century of plastic surgery has amply demonstrated, that full thickness grafts are usually indicated whenever Ollier Thiersch or thin split thickness grafts have proven unsatisfactory or inadequate.

In 1914 John Staige Davis described the superiority of using deeper skin grafts than those obtained by Reverdin's techniques (36) Whereas Reverdin's grafts included only a very thin smidgen of superficial dermis, the *"small deep grafts* or *Davis grafts* included at their centers almost the entire full thickness of skin with the deepest layers of dermis serving as an integral part of the graft. However because both the area thus grafted and the donor site when healed, have an unsightly pebbled or cobblestone appearance the Davis small deep grafts have largely been replaced by the use of pieces or sheets of *intermediate'* or *'thick split thickness skin"*

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The endocrine gland that most markedly affect the skin are those developed from the same embryologic layer (45)

Thus the posterior pituitary and the adrenal medulla prolong or intensify the effect of the sympathetic nervous control of skin blood vessels. Because invagination of the primitive ectoderm results in formation of the entire alimentary tract, it is not surprising to find dermatoses with gastrointestinal origins These have been mentioned for years by dermatologists as examples of disorders resulting from two organ systems that share a common embryonic derivation from the ectoderm

One of the first ectodermally derived glands to develop in the alimentary system is the parotid. Here again dermatologists have demonstrated the importance of these primitive derivations in describing a syndrome of certain central nervous system pathologic degenerations in which the sebaceous glands as well as the parotid gland undergo a modification of their normal activity (46) These diseases of various adult tissues and organs based on embryologic interrelationships assume great significance in an understanding of recently described mechanisms dealing with a human or animal sensitization to skin tissue or other tissue of ectodermal origin (47–49) Voisin and Maurer described a skin disease in rabbits who were grafted and injected with autologous and homologous skin and adjuvants (49) In the rabbit "the dermatosis was characterized clinically by a disseminated alopecia often distributed around the veins with thinning of the skin (49) Similar to this instance of disordered skin and hair both ectodermal structures Waksman (50) cites the examples of several human diseases (sympathetic ophthalmia the Vogt Koyanagi syndrome Harada s disease) in which there is simultaneous involvement of the uveal tract, the central nervous system and meninges the middle ear and the skin and hair (51 52)

The foregoing is a brief treatment of some of the embryologic factors that explain the multitudinous and extremely varied physiologic and pathologic relationships of skin This leads one directly into a brief discussion of the function of skin Gaines (40) summarizes the function of skin lucidly and briefly as follows

## SKIN FUNCTION

To these histogenetic and phylogenetic relationships must be added one final factor to wit the primitive ability of the cell to modify itself to meet conditions, and persistence of the primitive relationships in the human body This involves the defensive activities of the cells of the skin in the production of histamine, and in the reactions of the tissues in allergic phenomena and in infection or immunity It has been well said that as each cell carries on the functions of digestion, and of respiration and of reproducing itself so the individual cell carries some of the ability to protect itself

As to the skin as a whole, I cannot forbear to quote from two of our contemporaries, Medicine will learn to regard the skin as a highly differential parenchymatous organ of special significance in immunologic as well as in physical and chemical protection (53) "

It does not take a thorough understanding of physiology to recognize that the chief and perhaps first defensive barrier of the body against microorganisms is the skin itself The ability of skin cells to protect not only themselves but the body contents they envelop as well is quite remarkable. Kahn (54) demonstrated that different body tissues in the same individual have wide variations in their defensive capacity against known quantities of foreign antigenic material. The defensive capacity of a tissue to localize and "wall off" microorganisms or antigens (including homograft antigens) thereby preventing their spread throughout the body he termed the "localizing capacity" of the tissue. Skin as expected was shown to have a much greater localizing capacity than any other tissue.

The localizing capacity of skeletal muscle, for example, is approximately only 1/16 that of skin. Therefore, less violent rejection of skin homografts and thus their accompanying specific homograft antigens may be expected when the host site is skeletal muscle than when it is skin. This extraordinary localizing capacity is also possessed to a high degree by other surface tissues exposed to the external environment" with its ever present pathogens such as the mucosal tissue lining the throat (55) Its localizing capacity is chiefly an active function of skin-like cells.

Probably the most important passive function of skin on the other hand, is its ability to serve as a relatively waterproof envelope for the rest of the body It thus permits the underlying tissue cells and their aquatic-like surroundings to

survive, reproduce and function in a relatively normal saline fluid environment. This waterproofing can act favorably in a reverse manner—it permits the human body to be immersed in fairly concentrated salt water of the sea without loss of its own normal fluid balance or equilibrium and thus without shrinkage in a similar manner it permits the human body to swim or bathe in fresh water without swelling

Maceration of the dead surface layer of keratinous epidermal cells is prevented by a protective oil secreted by the sebaceous glands. Interestingly these glands are absent in the palmar and plantar skin and contrary to the general rule above surfaces of these areas will often swell following prolonged immersion in water unless a protective coating of oil is applied to them. Any oil found on the palmar skin however is actually contact oil originating from one's rubbing the face or from direct contact with some other oily surface. The passive protection of these dead epidermal cells is more apparent than real because as a layer it is constantly being replaced by the outwardly advancing movement of deeper viable basal epidermal cells that line the basement membrane. These basal cells cast off new cellular bodies that maintain a fairly constant thickness of the external keratinous layer as it is being brushed away by gradual desquamation and mechanical removal. In this sense it is really the deeper viable basal epidermal cells that protect and retain the fluid environment so necessary for the survival of all other underlying cells of the body

One cannot overemphasize the fact that all evolutionary antecedents of present day man came originally from an aquatic environment, and man himself today remains essentially aquatic He survives in a relatively hostile air environment, above water only because of his protective epidermal covering he might figuratively be compared to a sack of sea water his epidermis serving as the walls of the sack, or better still, to an aquarium in which the collective tissue cells of his body can live quite comfortably in a sea water environment. If this water is *drained away* as so frequently happens in thermal burn of the body the cells will die. Homer Smith (56) and others have declared that the salt content of interstitial fluid in the human body closely resembles that of the oceans several million years ago when the first primitive simple unicellular protozoan forms were evolving at the lowest rung of the phylogenetic ladder

In certain regions living cells of the body surface are unprotected by the horny keratinous epidermal layer *e g* cornea But even here a film of liquid is almost continually present to maintain the aquatic environment and protect the sensitive corneal cells.

The skin also serves as a barrier to the harmful effects of too much light, particularly sunlight by becoming pigmented the direct result of the activity of its melanoblast cells. It is not impervious to all substances, however and certain chemicals such as mercury can be absorbed through it directly into the blood stream This latter characteristic of skin is sometimes useful for treating local disorders by the topical application of various absorbable pharmaceuticals, *e.g.*, hydrocortisone ointment but it also constitutes a severe industrial hazard in some occupations where contact dermatitis can result from too much local absorption of an injurious agent, *e.g* synthetic dyes containing paraphenylenediamine (44)

Unfortunately skin offers only a very slight token resistance to x-rays and radium emanations its epidermal layers and epidermal derivatives or appendages may undergo malignant change when exposed either to prolonged sunlight over a period of many years or to any excessive amount of x-ray or radium

Skin is also very important in the regulation of body temperature. On cold days it acts as an insulation and on hot days it facilitates heat loss through perspiration and evaporation In perspiring the skin actually serves as an excretory organ. When an individual perspires too profusely however so much salt can be lost from the body surface that parenteral salt replacement becomes necessary to prevent heatstroke.

The skin is a manufacturing site where vitamin D the antirickets vitamin is formed wherever the skin is exposed to ultraviolet light. Children who do not have vitamin D in their diets will certainly develop rickets unless ultraviolet light in some form is permitted to bathe their skin and vitamin preparations are provided in their diet.

It would not be fitting to conclude this discussion of skin function without at least intimating the major role served by the profusion of specialized end-organs at various levels of the dermis for they are the receiving apparatus for sensa

*tions of heat, cold, pressure, and light touch* These end-organs apparently survive to some extent in full thickness skin autografts freely separated and then transplanted to another site in the same body although their recovery and function are slow and retarded. Pain is experienced as a sensation through specialized *end-organs* lying not only in the dermis but in the deep layers of the epidermis as well.

The foregoing functions are but a few of the remarkably numerous services of skin Many other specific functions are dealt with in subsequent sections devoted to its gross and microanatomy

## STRUCTURE OF SKIN

When choosing a donor site for skin grafts in the human being one becomes aware that skin varies considerably in characteristics such as texture, thickness, and elasticity depending upon its body location. In the realm of plastic and reconstructive surgery there is practically no skin area of the human body that cannot, if necessary serve as a donor site either for free grafts or for rotation flaps of skin.

In some areas of the body skin is especially thick taut, and horny *e.g* the soles of the feet, whereas in others it is thin, pliable and translucent, *e.g* the eyelids Where it covers soft parts such as the abdomen, skin tends to be flaccid and usually glides or moves in a fairly free manner but where it covers bony regions, as over the tibia it has a tendency to be relatively immobile and firm.

Skin is rough, smooth dry or moist depending upon the nature and volume of its glandular secretion and upon the degree and thickness of its superficial horny or keratinous layers. Its resiliency and tensile strength vary greatly with the body site involved, the age of the individual, and the individual himself In some areas it is almost hairless, *e.g* the posterior auricular skin in others it is quite hirsute and in still other sites it can often appear quite downy Skin color is determined by differences in thickness pigmentation and vascularity varying considerably from one individual to another (57)

Although many medical men with the possible exception of most plastic surgeons and dermatologists believe that skin is simple in its general structure it is probably one of the most complex organs of the human body The epidermis and its derivatives or appendages *e.g* hair follicles *sebaceous glands*, *sweat glands* etc are of ectodermal origin whereas the dermis is of mesenchymal or mesodermal origin. Specialized nerve endings in the dermis have their own highly specific parenchymal cell types essential for the perception of such sensations as heat, cold and pressure. The melanoblast another specific cellular type is found *concentrated in the* deeper epithelial layers of the epidermal ridges or rete pegs its function is chiefly that of producing the melanins that give skin its varied pigmentations.

As a tissue or organ unit skin consists of a series of strata or layers with only its epidermis and dermis being fairly well demarcated from each other by their histologic characteristics The *epidermis* is the most superficial, lying just above the connective tissue layer called the *dermis* or *corium*. The latter is histologically divided into two layers, a soft outermost *papillary layer* and a main, dense, deeper portion called the *reticular layer* Below the dermis lies a loose areolar layer the *tela subcutanea* or "subcutaneous tissue, which in some portions of the body is transformed into a more truly fatty tissue known as the *panniculus adiposus* (58) And finally the deepest skin layer only vestigial in the human organism, is the *panniculus carnosus* a discontinuous flat layer of skeletal muscle separating the skin from the rest of the body tissue it covers. In most animals the panniculus carnosus is very well developed, but its only visible remnant in man is the platysmal muscle layer of the skin of the neck (57) The tela subcutanea or subcutaneous tissue in man serves principally to adhere the overlying skin layers to superficial skeletal muscles and other underlying tissues.

Distributed widely throughout the dermis are smooth muscle fibers, the *arrectores pilorum muscles*. In some body areas these are *especially* numerous *e.g* in penile and scrotal skin.

As a rule skin tends to be thicker in males than in females but in the latter the subcutaneous tissue is more abundant. Skin is generally thinner on ventral or flexor surfaces of the body and thicker on dorsal or extensor areas. Over most of the entire body surface skin lies in well oriented furrows or grooves that are specifically arranged as cleavage lines of Langer or Langer's lines" (59) These lines usually indicate to the surgeon the direction in which the elastic tension of the skin and the underlying muscles are pulling A

knowledge of Langer's lines permits the plastic and reconstructive surgeon to provide his patient not only with the best possible functional result in any planned surgical incision, but the most satisfactory esthetic or cosmetic result as well. Incisions generally made at right angles to Langer's lines instead of parallel to them will often result in cosmetic and functional deformities e.g., scar contractions.

Skin has an especially rich supply of cutaneous sensory nerves that are single nerve fiber end organs of larger cutaneous nerve trunks coursing through the subcutaneous tissue. These large trunks break down into branches that divide and subdivide just beneath the dermis where

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plexus … turn supplies each dermal papillary body with a papillary vessel network or arcade.

Each dermal papilla is also supplied or drained by a meshwork of lymphatic capillaries. These collect into deeper lying branches forming a lymphatic network that lies under the arterial rete cutaneum in the subcutaneous tissue. The larger lymphatic vessels in this plexus are



FIG 50 Distribution of blood vessels in the skin *s* Subcutaneous tissue *r* Reticular layer of dermis *p* Papillary layer of dermis *R* rete cutaneum *Rp* rete subpapillare or subpapillary plexus (Modified slightly after v Brunn From A A Maximow and W Bloom A Textbook of Histology ed 4 W B Saunders Company Philadelphia 1944 )

equipped with valves. Eventually the largest of these vessels leaves the area it is draining by way of the tela subcutanea, and the blood vessels supplying that area do likewise (57)

### The Epidermis

The epidermis is called a stratified squamous epithelial covering for the entire body surface As such however only its most superficial layers are truly squamous or 'scale-like. Beneath them, the deeper epidermal layers consist of cuboidal columnar fusiform, or polyhedral shaped cells.

In most body areas the epidermis is approximately as thick as an average sheet of onion skin paper Histologically it is arranged in five layers that are not too distinctly separated but rather generally blend into one another as a result of a continuous proliferation and "scaling off" of cells from the deepest germinative or basal layer of epithelial cells These five layers are most characteristically seen in areas of the body where epidermis is thickest e.g the skin of the palms and soles where the outermost epithelial

cellular shape that are responsible for a histologic demarcation into the five separate epidermal layers just described.

The basal Malpighian cells of human skin are usually cuboidal cylindrical or columnar in shape and lie perpendicular to the external skin surface. Mitotic figures occur with great frequency in this layer Immediately above the Malpighian cells the cellular forms become more polyhedral in shape and thence become increasingly flat as they make their way out to the stratum granulosum where they are flattened in a horizontal position. The cytoplasmic borders of stratum spinosum cells are covered with very fine little spines that connect and intercross with similar spines of adjacent cells thus forming interdigitating bridges that fill the intercellular spaces. These spines or bridges account for the alternate terms for these cells namely 'prickle cells' or 'spiny cells." The prickles or spines are also called intercellular bridges' by many histologists

In the stratum granulosum, the granular cells have a rhomboidal shape in their deepest portions As this layer extends more toward the surface and the cells become more flattened, the cytoplasmic granules increase in number and the cellular nuclei gradually disintegrate and become very pale. At the same time the intercellular spaces become narrower and intercellular bridges become rather indistinct and shorter (58)

Under high power magnification the stratum lucidum consists of layers of very closely packed flattened cells which under low power magnification appear only as a pale, wavy stripe In most microscopic sections nuclei can no longer be seen in this layer

Stratum corneum cells are merely dead cornified, flat cells in which all intercellular bridges have disappeared In palmar and plantar skin, the cytoplasmic margins of these keratinous cells are covered with very small irregular spines and these probably account for some of the adherence and close contact they are able to maintain in these areas where they are especially compact It is in this cornified layer in most other body areas that desquamation of surface cells is constantly taking place with continual replacement of the desquamated cells by new cells progressing outwardly from the deeper basal layers

Although the five epidermal layers are quite distinct in most microscopic sections taken from palmar and plantar skin the epidermis of most of the rest of the body is much thinner and has a simpler structure with fewer recognizable layers. In many body sites for example, only two layers the stratum Malpighii and the stratum corneum can be detected the granular layer is frequently absent and when present, it generally consists of only one layer of cells in thickness *e.g.*, the skin of the shoulder

In most body sites the line of contact between epidermis and dermis is quite wavy and uneven. Only in the skin of the external ear the forehead and the midline of the scrotum and perineum does this junctional area appear as a straight line (58) In the rest of the body surface the outermost dermal layer is usually thrown up into a series of irregular ridges called *dermal papillae* and it is between these ridges or papillae that the lower or basal epidermal layers impinge or insert themselves as epidermal fingers or *rete pegs*

The dermal papillae separating each epidermal rete peg from its neighbor are highly vascular structures. Although epidermis itself has no blood vessels whatsoever it receives its nourishment from tissue fluid that makes its way into the intercellular spaces of the Malpighian layer from capillary arcades that lie in each adjacent dermal papilla or in the underlying dermis.

Skin receives its coloration or pigmentation principally from melanin granules that fill some cells in the basal layer of the stratum Malpighii. The melanin is manufactured or originates in cells called melanoblasts melanocytes or dendritic cells. In most histologic sections of white-skinned individuals these dendritic cells or melanoblasts are masked or are difficult to see because of the greater abundance of basal Malpighian cells. Melanoblasts are chiefly found in greater profusion in the epidermal rete pegs in white skin and they are less profuse in the basal epidermal cells that lie between the rete pegs (or on top of the dermal papillae)

In some manner as yet not clearly defined epidermal melanoblasts transfer their melanin to basal epidermal cells called melanophores Masson (62) believes that the melanoblast actually functions as a glandular type cell secreting its melanin pigment essentially by injecting it into adjacent epidermal cells through

its dendritic processes. Maximow (58) attributes skin color to three factors: 1) the inherent color of skin itself is chiefly a yellowish tinge; 2) melanin in the basal epidermal layers provides varying shades of brown; and 3) the underlying dermal vascular bed gives it a reddish hue. Any combination of these three colorations in an individual, therefore, will provide varying degrees of pigmentation. The melanin itself may range in intensity from yellow to a deep black in color (57). In the Negro and in heavily pigmented white skin, melanin is found in the cells of the stratum spinosum, stratum granulosum, and even the stratum corneum. In normally pigmented white skins, however, the greatest amount of melanin pigment is confined to cells of the stratum Malpighii. The fine granules of melanin pigment in these cells gradually disappear with the outward movement and proliferation of these cells such that the stratum corneum of a normal white-skinned individual has only a minimal, diffuse coloring attributable to the few remnants of melanin pigment that reach the surface. The deeper pigmentation of the Negro is caused by a much greater amount of melanin pigment in every epidermal layer.

Although it is not within the scope of this chapter to present a detailed histologic discussion, it should be mentioned here that the nuclei of epidermal cells have certain characteristics which help histologists and other research workers interested in skin grafting to determine the viability or survival of some skin homografts. Generally the shape of most epidermal cells will determine the shape of their nuclei. Most epidermal nuclei are spherical in form and occasionally some epidermal cells have not one but two nuclei. Recently Barr (63) demonstrated that the nucleus of female cells, *e.g.*, female epidermal cells, is characterized by a very distinct mass of chromatin in the form of a planoconvex nucleolus which adheres itself to the inner surface of the nuclear membrane.

This chromatin mass or nucleolus, called the "sex chromatin," is characteristic only of female cells and is not seen in the nuclei of typically male epidermal cells. The advantages of using this histologic "marker" to identify permanent survival or even temporary survival of a female skin homograft placed on a male patient or a male skin homograft placed on the female patient seem obvious. Peer (64) used this very method to determine the survival of a female skin homograft taken from a mother and placed on her infant son.

When the sex chromatin mass lies at the periphery of an epidermal cell nucleus, it can be quite readily identified after considerable microscopic practice and experience. Even if it does not lie in this typical position, an experienced investigator can fairly well recognize it in an atypical site. When present, it stains with hematoxylin and is Feulgen-reactive. In contradistinction to other chromatin particles found in the nucleus, it is usually larger, has a planoconvex shape, and in most instances lies against the nuclear membrane at the very periphery of the nucleus. As a point of further contrast, the average typical nucleolus of an epidermal cell is spherical in shape rather than planoconvex, and is Feulgen-negative. Any difficulty in distinguishing the sex chromatin mass from a normal nucleolus can almost be ruled out at once by employing the Feulgen test.

In most epidermal cells of the deeper layers *mitochondria* are usually abundant. In basal columnar cells they are generally situated above the nucleus and below it, whereas in overlying squamous cells of the more external layers, mitochondria are usually found surrounding the nucleus.

In all viable epidermal cells the *Golgi network* or apparatus is always present and with rare exceptions it lies above the nucleus. In some cells it also surrounds the nucleus in the shape of a loose basket type meshwork. Cowdry and Scott (65) have demonstrated the Golgi network by staining epidermal cells with neutral red employing the supravital method. Since the Golgi network lends itself to supravital staining, it, too, like the sex chromatin mass, becomes important in any studies that attempt to demonstrate the viability or survival of cells transplanted in skin homografts.

Parat (66) was probably the first physiologist to demonstrate that the Golgi network is actually a series of lipid vacuoles. In staining a specimen of so-called "viable" rat epidermis, therefore, a neutral red supravital stain will reveal the Golgi network of Malpighian epidermal cells to be a compact vacuolar mass lying above the nucleus. Sometimes this network will lie on either side of the nucleus and parallel to it in these basal epidermal cells. In cells lying immediately above the Malpighian layer this vacuolar system will stain as densely as it does in the

basal cells. In the stratum granulosum however it shifts its position quite often lying beneath the nucleus and will seem less dense and more dispersed. But in all of these cell layers as far as the stratum granulosum, it usually lies in close proximity to the nucleus of the cell (66)

In general the smaller vacuoles of the Golgi network stain more readily with neutral red than the larger vacuoles. In the stratum granulosum the larger vacuoles are usually found in a more basal position, juxtaposed along both sides of the nucleus whereas the smaller vacuoles are found in a higher position in the superior portions of the cell. As the cellular layers of the epidermis advance outwardly and keratinization becomes a factor the vacuolar Golgi system disappears (66)

### The Dermal Epidermal Junction

Montagna (57) in his excellent compendium of the most up-to-date findings on the structure and function of skin emphasizes that one factor contributing to the strength of the union or adherence of epidermis to dermis seems to be the interpositioning or interlocking of epidermal rete pegs with dermal papillae. Epidermal regions subject to a maximum of shearing forces, *e.g.*, soles and palms, are characterized by very well developed undulating epidermal ridges and dermal papillae.

An even firmer union is provided by delicate protoplasmic processes that extend out from stratum Malpighii cells across the *basement membrane* into the dermis. The basement membrane *itself* has recently proved to be more complex than histologists of past years apparently thought Electronmicroscopy reveals that this membrane is actually a very intricate argyrophilic meshwork through which the protoplasmic or cytoplasmic processes of basal epidermal cells protrude into the dermis

Cytologists believe that the dermis in a like manner sends out very delicate elastic fibers which insinuate themselves between the basal epidermal cells Thus an interlocking of epidermal cytoplasmic processes and dermal elastic fibril when compressed tightly together forms the basement membrane (57) This interlocking is a very intimate one and Montagna believes as do many other histologists that one of the principal functions of dermal elastic fibers might be to anchor down the epidermis" (57)

This supposition is borne out by use of trypsin which is believed to contain a specific enzyme elastase to separate the epidermis from the dermis (67) Billingham and Reynolds (68) demonstrated that sheets of pure epidermis and epidermal cell suspensions prepared by incubating skin shavings in buffered trypsin solution could be successfully autotransplanted. Interestingly they suggest that use of these epidermal cell suspensions might offer a practicable method of achieving a high degree of epithelial coverage on an extensive area of full-thickness skin loss for the minimum expenditure of intact skin' Trypsin in comparison to elastase *per se* or collagenase, apparently provides the quickest cleavage of epidermis from dermis with the least concentration of agent required. An elastase of some type is undoubtedly one of the constituents of the trypsin solution and it is probably an elastase that dissolves the delicate but strong elastic fibers normally uniting epidermis to dermis

Although cytologists are still in conflict regarding the exact nature of the basement membrane—is it chiefly epidermal or dermal in structure—for the purposes of this chapter it is enough to describe it briefly in order to differentiate it from the "pseudo-basement membrane" seen in special reticular fiber staining of skin *e.g* Laidlow's stain (69) Caby (69) in France used Laidlow's stain as a test of viability of a large skin homograft two and a half years after it was placed on a 3-year-old girl whose mother served as the donor This stain brings out quite distinctly the heavy staining qualities of reticular fibers which lie just underneath the basement membrane in surviving grafts whether autografts or homografts The intensity of the stain gives them the appearance of another undulating dark line lying immediately *beneath the basement membrane*. This dark line or "*pseudo-membrane*" is not seen in any area healed by 'scar tissue epithelium and dermis when similarly stained. Caby concludes, therefore that the presence of a "pseudomembrane in a skin biopsy taken of a grafted area months or years *after* the original grafting procedure is clear evidence of survival *of this* graft In Caby's own case, replacement of the mother's homograft by the *normal* processes of wound healing and scar tissue *formation* that occur whenever a homograft sloughs did not take place. Thus survival of the homograft two and a half years later was implied (69)

### The Dermis

The dermis is divided into two separate layers—a superficial, soft *papillary layer* and a deeper denser and more coarse *reticular layer* In general these two layers cannot be abruptly or specifically separated from each other

The papillary layer is composed of a meshwork of fine fibrous tissue containing delicate elastic and collagenous fibers in which great numbers of capillary networks and arcades predominate This layer serves not only as a bed for the terminal blood supply of the skin but also contains the sensory organs of touch The deeper reticular layer consists chiefly of much denser rather coarse closely interlaced collagenous fiber bundles that branch out in many directions but are essentially oriented in a direction that roughly parallels the skin surface. Only in a few body sites are these collagenous fibers actually arranged perpendicularly to the skin surface.

The perpendicular fibers are usually carried down into the subcutaneous tissue where they branch out loosely and incorporate themselves into the fatty tissues found in this layer forming fibrous bundles between and around fat lobules called *retinacula cutis* (57) Collagenous bundles in the papillary layer are more loosely arranged and are much thinner in quality

Dermal elastic fibers form a rather thick, abundant network that lies between the collagenous bundles tending to be especially predominant in regions surrounding hair follicles sebaceous gland and sweat glands Elastic fibers in the papillary layer are much thinner and in the dermal papillae themselves are arranged in a continuous fine elastic meshwork that lies just under the epithelium

In addition to the collagenous bundles and elastic fibers of both dermal layers one finds the ground substance an amorphous semifluid substance that fills the intercellular spaces between these fibers The papillary layer contains a relatively larger amount of ground substance than the reticular layer because there is a greater density of collagenous and elastic fibers in the latter

Throughout both dermal layers a reticular fiber network also exists These fibers are especially dense in the uppermost layers of the dermal papillae lying just beneath the epidermis. In the latter region they help to form either the basement membrane itself or at least an integral part of it. Reticular fibers like elastic fibers are especially abundant in and around the hair follicles sweat glands and any extensions of these skin appendages. Deeper in the dermis reticular fibers are prominent only in the area of blood vessels and in the deeper fatty layers of the subcutaneous tissue. They encase each fat cell of the panniculus adiposus in a basket-like fashion and envelop the blood vessels in the subcutaneous tissue in a similar manner

Most bodily connective tissues and the dermis is no exception, contain fibroblasts whose shapes are usually determined by the area in which they find themselves. Thus in the closely compressed collagenous bundles of the reticular layer fibroblasts tend to be equally flat and compressed, quite long and very thin In the more loosely organized connective tissue of the papillary layer on the other hand, they quite often resemble mesenchymal cells and are usually much larger and fatter

The dermis also contains histocytes which in most microscopic sections resemble fibroblasts so closely that it is difficult to differentiate between them. Because histocytes have a tendency however to accumulate wherever they can ingest foreign matter or particles they can be more easily observed wherever foreign particulate matter is present in the skin. In general there are two types of histocyte, the large and the small. Large histocytes will ingest or phagocytize foreign particles of various sizes but small histocytes can cope with only small particles in phagocytosis. If some of the foreign particles are too large for either form of cell several histocytes will act in combination surrounding the particle by apparently fusing themselves into so-called foreign-body giant cells. The latter are actually multinucleated plasmodium like masses (57) they are especially numerous at the host graft junction between a skin homograft and its adjacent or underlying dermal host-bed (70)

### The Subcutaneous Tissue

The *tela subcutanea* or subcutaneous tissue consists chiefly of a loose connective tissue containing many interspaces As such it is thought to be a mere continuation or a loose extension of dermal reticular fibers Its relatively few elastic or collagenous fibers course over directly into the dermal reticular layer in a direction that

roughly parallels the skin surface (58) In body sites where the skin is especially flexible, fibers of subcutaneous tissue are very scanty But in the soles and palms where the skin is not very flexible, its fibers are very thick and quite numerous helping to bind the tela subcutanea intimately to underlying structures.

Variable amounts of fat deposition or fat cell development are seen in the subcutaneous tissue layer depending upon the body area involved and the nutrition of the organism. The *panniculus adiposus* of the abdominal skin area for example will reach a thickness of several inches in some obese individuals. A fatty deposition of this magnitude would be quite impracticable, however in regions such as the eyelids or penis, where the subcutaneous tissue layer contains no fat cells at all (58)

In general, the subcutaneous tissue layer seems to serve principally as an area which either carries or is penetrated by nerve trunks and large blood vessels. As such it also contains many nerve endings responsible for the sensations of deep pressure and proprioception.

## SKIN APPENDAGES

The principal skin appendages—hair follicles sebaceous glands, and sweat glands—arise primarily from the epidermis. During the course of embryologic development the latter invaginates into the dermis in countless areas of the body creating intradermal, epithelial lined structures. The survival of deeply situated hair follicles, sebaceous glands, and sweat glands in an area of third degree burn in which the overlying epidermis and upper dermal layers are completely destroyed accounts for the often remarkable resurfacing of severely burned areas with epidermis originating from proliferative outgrowths of these intradermal epithelial structures.

### Sweat Glands

Sweat glands are simple tubular structures usually coiled in shape that lie in the deeper dermal layers but sometimes are found deep in the subcutaneous tissue *e.g.* circum-anal and axillary areas. Their excretory ducts are narrow unbranched tubes that pass up through the dermis from the coiled glandular secretory bodies" and out through the epidermis excreting their transparent watery sweat through individual pores lying on the skin surface Occasionally their excretory ducts empty just above the opening of the sebaceous ducts which normally secrete into the neck of the hair follicles.

In the coiled mass of each gland, the principal cell form is a single layer of truncated pyramidal or cubical cells interpositioned between myoepithelial cells. The latter are flattened spindle-shaped cells each containing an elongated nucleus and a cytoplasm filled with longitudinal fibrils Contraction of these myoepithelial cells apparently helps to discharge the secretion of the glandular" cells lying adjacent to them (58) The excretory duct is lined by a two-layered thin epithelium the basement layer cells having abundant mitochondria and fairly large nuclei. The external or lumenal layer of epithelium is characterized by cells whose cytoplasm is essentially a refractile cuticle.

The lumen of the excretory duct in the epidermis becomes a simple intercellular channel lined by epidermal cells concentrically arranged as such, it has no true wall of its own The duct becomes twisted spirally as it passes up through the stratum Malpighii and is especially twisted in the less flexible, keratinized zone of the stratum corneum (58)

Skin autografts are usually quite dry for a moderate period of time following their transplantation or at least until their sweat gland function is restored. This dryness is a direct result of complete severance of the free skin grafts from their original nerve system innervation with a concomitant absence of the lubrications normally supplied by sweat and sebaceous glands Until this lubricatory function is restored most plastic surgeons recommend the application of olive oil lanolin, or other bland ointments to the grafted skin areas.

### Sebaceous Glands

The sebaceous glands are saccular or alveolar in type. They are usually found immediately adjacent to hair follicles and have their excretory openings in the neck of the hair follicle along the hair shaft The secretory portion of these glands consists of lobules or rounded sacs resembling in many instances a bunch of grapes. The lobules are filled with fat droplets and other fatty detritus mixed with horny scales this the result of a proliferative desquamation and cellular destruction of a single layer of thin cells with round nuclei lining the alveoli The cytoplasm

of these cells gradually becomes distended with fat droplets and as the cells proliferate toward the center of the alveoli becoming larger and polyhedral in shape their distended cytoplasm tends to break down at about the same time that their nuclei shrink and finally disappear The mixture of fat droplets fatty detritus, and horny scales becomes the "sebum" or sebaceous secretion of the gland (58)

The short excretory duct is lined by a stratified squamous epithelium which is continuous with either the external root sheath of the hair or the stratum Malpighii of the epidermis. The fatty or oily sebaceous secretion like that of the sweat gland serves not only as a lubricant for the hair shaft but also helps to keep the overlying skin supple relatively impervious to moisture and protected against continued friction

Sebaceous glands are distributed over most of the body surface with the exception of the palms and soles. In general each body hair is equipped with one or more sebaceous glands which frequently lie in the very apex of an acute angle whose two arms are formed on one side by the hair shaft and on the other by an arrector pili muscle coursing in an oblique direction toward the skin surface. Situated snugly in this apex the gland is affected by any contraction of the arrectores pilorum muscle which compresses it between the muscle body and the hair shaft and thus helps to discharge some of the sebaceous contents along the hair shaft itself Destruction and secretion of the glandular cells are followed by a regenerative multiplication of the cells lining the alveolus or the body of the gland.

### Hair

Hairs are essentially a collection of dead, keratinized cells that have been compactly cemented together in the form of threads (57) They develop from epidermal invaginations or tubes called hair follicles which lie at various depths in the dermis and subcutaneous tissue, depending upon the state of progression of the so-called "hair-cycle" (71)

Skin of most of the body surface is covered by hair which is usually a soft "downy" type except in those areas where the hair shaft is longer and coarser *e.g* the scalp eyebrows and pubes There are no hair follicles however in eyelid skin nor are any to be found on the palms soles lips prepuce glans penis volar and lateral surfaces of the fingers and toes, etc

The histology of hair is fairly complex, but Montagna (57) presented a lucid description as follows

> Hairs grow out of tubes of epidermis sunken into the dermis the *hair follicles* Beneath the surface of the skin the hair is encircled by the *follicle* which is a sleeve of epithelium continuous with the surface epidermis At the base hair follicles are dilated into an onion shaped region called the *bulb* the greatest diameter attained by the follicle is through the bulb. The bulb is hollowed out at its base into a cavity which is completely filled with a packet of loose connective tissue called the *dermal papilla* Hair follicles are composed of an outer root sheath and an inner root sheath The thickness of the outer sheath is directly proportional to the size of the hair follicle and is very thick in the follicles of large hairs The inner root sheath is composed of three concentric layers an outer layer one cell in thickness which rests against the outer sheath Henle's layer a middle layer two or more cells in thickness Huxley's layer and an inner layer which rests against the hair the cuticle of the inner sheath Hair follicles are surrounded by a connective tissue sheath composed of an inner circular and an outer longitudinal layer The connective tissue sheath is continuous above with the papillary layer of the dermis and at the base of the follicle with the dermal papilla These structures are contiguous and the connective tissue sheath and the dermal papilla of hair follicles can be considered as parts of the papillary layer of the dermis (57)

Thus it can be seen that the usual three epidermal layers—corneum, granulosum and Malpighii—dip down into the hair follicle and are contiguous with the layers of the follicle. At the neck of the follicle, where the excretory duct of the sebaceous gland usually empties the surface epidermal layers directly adjoining the hair shaft become thinned out into only one layer the stratum Malpighium. Continuing down into the deeper portions of the hair follicle it is this Malpighian layer which forms the external or outer root sheath of the hair (figs 51 52)

Every hair goes through a period of active growth and mitotic division ("anagen") with enlargement of the hair follicle followed by a period of transition ("catagen") and terminated by a resting or quiescent period ("telogen") These three periods are known collectively as the "hair cycle" (57 71) or "skin cycle" (72) A resting hair follicle for example is in its simplest form extending no deeper than the

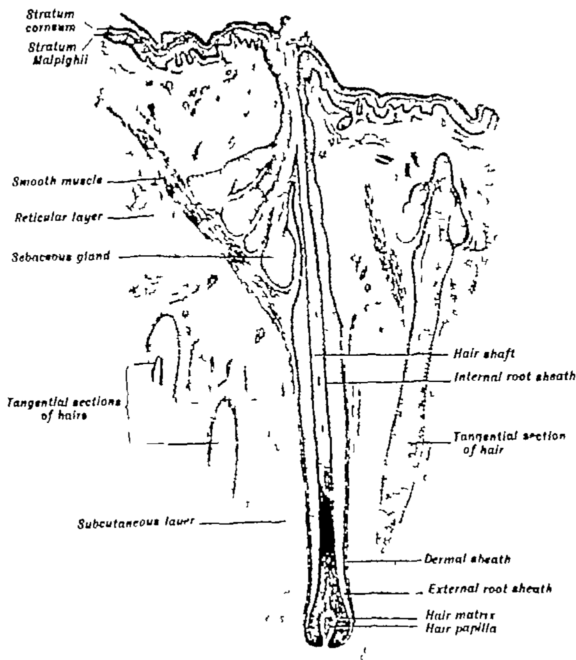



Fig 51 Scalp of a man Root of a hair in longitudinal section 32 × After Schaffer (From A A. Maximow and W Bloom A Textbook of Histology ed. 4 W B Saunders Company Philadelphia 1944)

dermal reticular layers With the beginning of active growth or "anagen however the hair bulb gradually dips down through the dermis and extends into the underlying subcutaneous tissue or panniculus adiposus at its period of maximum extension it may lie fairly deep in the panniculus. As the regressive stages of catagen begin, those hair elements that have extended themselves down into the panniculus adiposu degenerate and a new hair cycle" begins with the hair bulb once again lying entirely within the dermis Ballantyne and Converse (71) and Randall and Dushoff (72) have similarly demonstrated that the survival time of skin homografts transplanted in animals (rats and mice, respectively) varies considerably depending upon the phase of the hair cycle existing at the time of transplantation

## THE BEHAVIOR OF AUTOGRAFTS AND HOMOGRAFTS

Any orthodox approach to a chapter on skin transplantation" must naturally deal with the

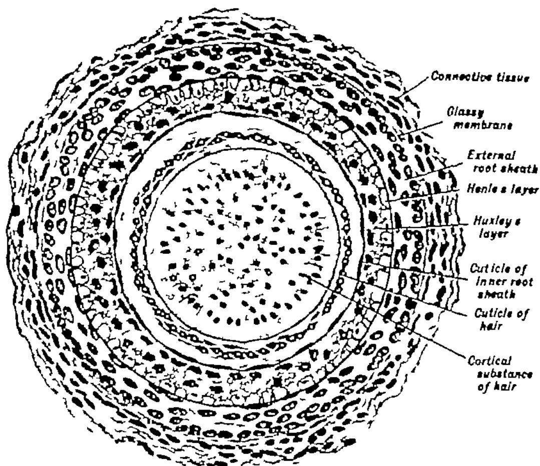



FIG 52 Cross section through a hair follicle in the skin of a pig embryo ×375 (From A A. Maximow and W Bloom A Textbook of Histology ed 4 W B Saunders Company Philadelphia 1944)

gross and microscopic behavior of skin autografts. Peer (38) rightfully emphasizes

'Current interest in homografting has a tendency to lead investigators to neglect the autograft It is our belief that the behavior of autogenous grafts is an important yardstick in evaluating the fate of similar homogenous transplants

Since the behavior of skin autografts is such that, under good surgical conditions and with a modicum of careful postoperative treatment, any autograft should take and survive permanently in most instances the skin autograft can serve as a control or frame of reference for the altered or inconsistent behavior of skin homografts

Skin homografts are almost always employed as emergency *temporary* dressings for the extensively burned patient. It is their limited durability however which poses the major problem in their use and is the subject of much study in both clinic and laboratory The homograft takes well initially It becomes vascularized from the host-bed in a manner similar to that of permanent autografts and—for a time—seems to be accepted by the host environment. The period of acceptance has been found to range between 6 to 9 days in human volunteers (70) up to periods of many weeks in burned individuals (11–15)

But at some point in its course—approximately from the seventh to the ninth day in the nonburned human volunteer the graft undergoes a sudden change. The re-established blood flow in its dermis becomes shut off as it were by a progression of events that includes 1) stasis or stagnation of blood flow within the graft vessel then 2) vascular thromboses 3) increased vascular permeability 4) rupture of the vessel walls 5) spillage or diffusion of erythrocytes into the perivascular tissue spaces of the graft 6) desquamation of the overlying epithelium and finally 7) ulceration and erosion of the graft dermis Following these phenomena there is

usually left behind on the host bed a collagenous 'dermal pad' of the graft which is ultimately absorbed and replaced by the host's own tissues.

This then is the 'problem' of the skin homograft. It is pertinent to consider the mechanism or mechanisms that determine the permanent survival of skin autografts but cause the ultimate rejection of most skin homografts.

The differences in histologic behavior between the two types are largely a matter of leukocytic responses and vascularization. The autograft usually calls forth only a minimal polymorphonuclear and lymphocytic response from the neighboring host tissues during the first ten days postoperatively while the homograft evokes a major lymphocytic and plasma cell invasion approximately five to ten days postoperatively and subsequently between the tenth and twentieth postoperative day, also shows an eosinophilic response.

Although these leukocytic responses are fairly well described and almost uniformly consistent, the methods of re-establishing a blood supply for both free skin autografts and homografts are still a subject of controversy. For many years some workers claimed that homografts were never revascularized (see review by Converse and Rapaport (73)). Using a simple technique involving the injection of India ink however Scothorne and McGregor (74) in 1953 demonstrated conclusively that homografts like autografts were definitely revascularized prior to their rejection the revascularization being completed between the fourth and fifth postoperative days.

Their studies of rabbits using full thickness skin grafts were substantiated by the work of Converse and Rapaport (73) and many other researchers. The findings described in the following sections apply to *full thickness* skin grafts in human beings but they do not differ essentially from results with human split thickness grafts. In general there is no observable difference in the development and vascularization of skin autografts and homografts prior to the onset of the homograft rejection phenomena in the human host. During the first five to seven postoperative days both gross and microscopic behavior of the two types are almost identical.

### Gross Behavior of Autografts and Homografts

A description of the comparative gross behavior (73) of these two types of grafts will be followed by a comparison of their microscopic behavior (70)

'Each graft had a blanched appearance at the time of application persisting for a period of approximately 24 hours Occasional patches of pink appeared in the graft by the second day During the first 48 hours accompanying the initial period of healing an area of *primary erythema* appeared in the skin surrounding the graft the surface of the graft presented a shiny appearance with no evidence of edema The graft assumed a pink tint on the third or fourth day and the surrounding erythema and edema disappeared (73)

From the sixth postoperative day onward the gross appearance of the autografts and homografts changed.

#### *Autografts*

'Surface epithelium desquamation and active regeneration and merging of graft and host epidermis occurred on the sixth or seventh day The surface of the graft eventually became covered with shiny new epithelium which increased in thickness and lost its shiny character Between the tenth and twelfth day the graft became pale and by the twentieth day resembled the color of the surrounding skin (73)

#### *Homografts*

After the disappearance of the primary erythema, a halo of *secondary erythema* and edema surrounded the homograft by the seventh day This change attained its maximal intensity on the eighth or ninth day The pink color of the homograft deepened after the sixth day became cherry red in color and appeared cyanotic by the ninth day The homograft showed only a minimal degree of desquamation and merging with the surrounding skin the autograft at this period displayed marked activity

The graft became swollen as the color changes occurred rising above the surface of the surrounding host skin. It acquired a pneumatic appearance which increased to the ninth or tenth day

Graft desiccation and escharification followed leaving a dry and *opaque surface on the twelfth or* thirteenth day The graft a brownish eschar by the fifteenth day became sloughed by the twentieth day leaving a dermal pad in the host bed (73) (See figs 53–54 for a progressive sequence of the homograft rejection phenomena )

### Microscopic Behavior of Autografts and Homografts

Microscopic data obtained by Rogers ET AL. in 1951 in non related human male volunteers (70)




Fig 53 Behavior of a first-set skin homograft in the human host 1 Fifth postoperative day Initial take 2 Ninth postoperative day dermal hemorrhage and epithelial desquamation 3 Twelfth postoperative day epithelial desquamation denudes the raw dermal bed 4 Fourteenth postoperative day first appearance of the dry necrotic dermal pad at 12 o clock 5 Nineteenth postoperative day: further progression of dry gangrene in the dermal pad 6 Twenty first postoperative day the dermal pad is entirely gangrenous 7 Twenty-sixth postoperative day gradual erosion of the dermal pad. 8 Thirty-second postoperative day gradual erosion of the dermal pad 9 Thirty fourth postoperative day note the contraction of the wound edges (From B O Rogers The genetics of skin homotransplantation in the human Ann New York Acad Sc 64 741 1957 )

demonstrate the changes between *full thickness* autograft and homograft behavior noted by others, notably by Medawar in his basic studies of graft behavior in rabbits (8–9)

*Autografts*

From the third to the tenth postoperative days the autograft dermis had a characteristic vascular congestion All of the arteries capillaries and dermal veins were greatly distended with closely packed red blood cells, making the vessels stand out clearly from their surrounding collagen bundles. Newly-ingrown host vessels of small caliber invaded the graft at all host-graft junctions, but were especially numerous in the granulation tissue of the underlying host-bed, where they penetrated the dermis of both auto-

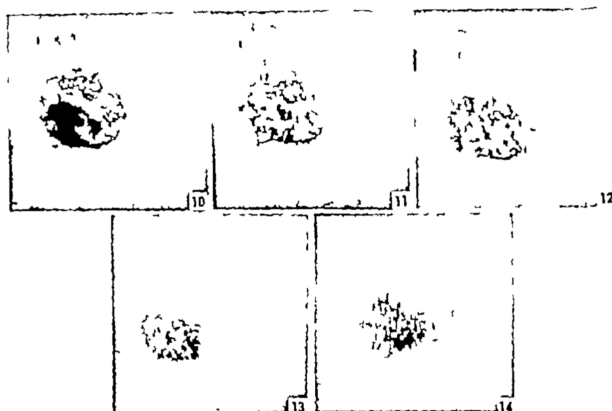



FIG 54 Behavior of a first set skin homograft in the human host (cont d ) 10 Thirty-eighth post operative day some of the dry gangrenous dermal pad has sloughed 11 Forty first postoperative day the entire dry gangrenous portion of the dermal pad has sloughed 12 Forty-eighth postoperative day remnants of the dermal pad on the raw granulating bed 13 Fifty first postoperative day the con tracted wound edges have almost completely cast off the dermal pad remnants 14 Fifty fourth post operative day the dermal pad of the original homograft has completely sloughed (From B O Rogers The genetics of skin homotransplantation in the human Ann New York Acad Sc 64:741 1957 )

grafts and homografts as perpendicular sprouts There were no significant numbers of leukocytes present in the graft dermis with the exception of a few polymorphonuclear cells occasional lymphocytes and mononuclear cells Some multinucleated giant cells were seen at the host graft junction, probably in response to the suture material in this region and to some of the prod ucts of cellular damage resulting from the sur gical trauma itself The cell population of the granulation tissue lying between the graft and the host-bed was fairly sparse and homogeneous consisting chiefly of a few lymphocytes occasional plasma cells polymorphonuclear and mononu clear cells (70)

*Homografts*

Early changes in the homograft dermis be tween the first and fifth postoperative days largely paralleled those of the autografts so that it was difficult to detect any difference between the two. During this time all homograft dermal vessels were greatly engorged and re mained so during the degenerative events that resulted in their complete destruction. As in autografts, leukocytic infiltration during the first five to seven days was generally very slight in the body of the homograft. There was how ever usually a more pronounced accumulation of polymorphonuclear cells at the host-graft junction during the first five days. From the fifth to the tenth postoperative days however, in contrast to autografts, a gradually increasing number of lymphocytes and plasma cells invaded the graft the host-graft junction and the im mediately neighboring host tissues (70)

This small round cell infiltration, as it gradu ally grew in intensity was concomitantly asso ciated with more drastic changes in the homo graft on or about the seventh to the ninth postoperative days These changes con isted of 1) the complete disappearance of all old graft vessels and newly ingrown host capillaries 2) the disappearance of all fibroblasts endothelial

and other cells previously seen in the homograft dermis and 3) the sloughing of the graft's dead or dying epidermis (70) (See figs. 53-54)

In some patients engorged graft vessels seemed to rupture before the red blood cells within were hemolyzed this resulted in many areas where extravasated erythrocytes spilled out into the graft dermis, filling the spaces between collagen bundles and converting the area within a day or two into a localized site of intense hemorrhage and focal gangrene (70) From approximately the tenth postoperative day and onward the histologic differences between autografts and homografts were even more pronounced.

*Autografts*

By the tenth postoperative day new connective tissue was being laid down to join host and graft dermis In the host-graft junction new blood vessel formation was very active by the tenth day but as yet these new sprouts penetrated only a very short distance into the autograft itself. From the fourteenth to the fifteenth postoperative days, however new host vessels extended throughout the length and breadth of the autograft. At first these new vascular sprouts followed the course of old graft vessel bundles and thus extended deeply between the collagen fibers of the graft dermis. As this process of final revascularization became firmly established the new vessels from the host-bed and margins then seemed to grow into the graft in an independent fashion, without the need of old vascular channels to guide them (70)

During the interval between surgery and this revascularization many graft dermal cells lost their affinity for histologic stains so that many reticular and papillary portions of the graft dermis appeared to be almost cell free. With the revascularization and its accompanying return of endothelial cells and capillaries the graft's dermal cells—fibroblasts macrophages, and amoeboid wandering cells—made their reappearance within the old elastic and collagenous fiber reticulum of both the papillary and reticular layers It is not clear whether these dermal cellular components proliferate from surviving graft cells or emigrate to the graft along with the endothelium of new ingrowing capillaries. Cellular survival *versus* cellular replacement in autografts is still a controversy brought recently into revived debate by Peer (38) (see detailed discussion in section on *Cell Survival vs. Cell Replacement*) In any event the non-cellular components of the autograft can and apparently do survive the ischemia to which they are subjected during the period between grafting and vascular invasion by the host vessels (70)

The fibrous dermal elements, collagen and elastic tissue were not replaced but persisted to form new components of the dermis of the grafted area. This dermis gave the autograft every appearance of being normal skin (dermis and epidermis) by the eighteenth to the twenty fourth postoperative days.

*Homografts*

As far as the homograft epidermis was concerned, a large number of epidermal cells were dead by the third to the fifth postoperative days and almost all epidermal cells were moribund by the tenth day The formation of a new marginal epidermis began by the tenth postoperative day and was observed as a young, actively mitosing layer of cells interposed between the old dead or dying homograft epidermis and the graft dermis. It apparently arose from the marginal host epithelium, since its continuity with this layer was easily seen in serial microscopic sections, and its thickness diminished as it coursed toward the center of the homograft (70)

With the radical changes that occurred in and about the homograft dermis between the seventh and the ninth day and twentieth postoperative day there was a complete desquamation of the original homograft epidermis. It usually sloughed off as a brownish eschar or a wet scab between the tenth and twentieth days leaving behind a whitish dermal pad the only remnant of the original homograft. Following the epidermal slough, a new invasion of host epidermis proceeded more rapidly from the graft margins It was still a slow process, however and appeared to be held in check by the degenerative changes which were still taking place in the underlying graft derml Epidermal ingrowth kept pace with the clearing away of underlying necrotic graft dermis and the invasion of this area of "repair" by new host blood vessels. The host epidermis frequently grew over bands of the graft's collagen fibers that were temporarily incorporated in the regenerating dermis Sometimes the advancing host epidermis attempted to undermine the remains or vestiges of the graft's dermal pad (70)

Accompanying these epidermal ingrowths and following the violent homograft rejection phenomena other reparative processes immediately set in. Among these were 1) hyperemia and hyperplasia of host tissues near the graft 2) marked activity in the newly formed scar tissue between host and graft, including new capillary formation clearance of debris by multinucleated giant cells and macrophages, and later the formation of new collagen fibers by numerous emigrating fibroblasts 3) the outgrowth of host epidermis (as just described) bridging the distance between native and foreign tissues and "attempting" to continue right over the graft (an overgrowth limited by the extent to which the new capillaries could penetrate the tenacious graft dermis) and 4) revascularization and clearance of graft collagen elastic fibers, and other dermal debris. This entire repair process occurred very slowly on an advancing front from the host which contained numerous capillary sprouts active giant cells and macrophages and numerous eosinophils. The activities of this advancing front resulted in the removal of graft dermis by a process of loosening collagen bundles with a subsequent lysis or digestion of their fibers coupled with a *pushing back if not* an actual breaking down or digestion of the graft's tough elastic fibers.

The advance of this host scavenging front into the "dermal pad" was often so slow that the "pad" became even more compact. It usually consisted of an outer layer of collagen fibers only and an inner layer of collagen bundles separated by necrotic cellular debris. Occasionally the advancing front bypassed an island of this graft dermis which was then later extruded through the new overlying epidermis or was cleared away by a type of rear guard phagocytic action In some cases the dermal pad was cleared away by the penetration of tongues of the scavenging front. In most cases, however the pad became impenetrable and so was undermined by the undergrowth of host epidermis it then sloughed either in layers or as an entire unit (see figs. 53-54)

As a rule the result of all this host activity left very little of the original homograft structure intact beneath the advancing host epidermis when healing-over eventually occurred nothing but a few wisps of old graft collagen fibers remained and these were ultimately absorbed digested etc

## THE PROBLEM OF SKIN HOMOGRAFTS

### Summary of Status of Skin Homografting

The first two pages of a comprehensive summary of the status of skin homografting by Conway and Stark in 1954 (76) will now be quoted almost in their entirety Although many advances have been made since this report it covers most of the essential highlights in the problem of skin homografts. As a framework for subsequent reference a network of brackets [ ] has been inserted by the present author in the body of the quoted passage The number enclosed in each bracket corresponds to a numbered section of text in which the topic under discussion is dealt with in more detail. The numbered sections follow the quotation and are for the convenience of the reader who may wish to make abbreviated reference to the problems of skin grafting Space at hand does not permit detailed treatment of all the problems of skin grafting such is presented however in a forthcoming book by the present author (B O.R.)

*Reports in recent years regarding the success*ful homotransplantation of tissues such as bone and artery have created confusion in the understanding of the entire subject of grafting of tissue from one individual to another in the same species. It has long been taught that cartilage cornea and lens may be homotransplanted successfully and this success has been attributed to the physiologic common denominator of these three tissues They lack anatomic vascular trees their tissues have low metabolic rates and they survive by nourishment from the intercellular fluids of their new host In contrast preserved tissues such as artery and bone are transplanted not as living tissues but as specific human anatomic structures which eventually are replaced by tissues developed by the repair processes of the host The latter are referred to erroneously as grafts In order to clarify this confusion Longmire has differentiated between the two types of homotransplant and has coined the separate terms homovital and homostatic grafts (77)

Four excellent reviews (11 78-80) of the literature upon homologous grafts were published in 1950 and 1951 They did much to introduce rational thought and criticism to a subject beleaguered by clinical observations without microscopy experimentation without controls case reports without follow ups and opinions bereft of logic

In the decade from 1940 to 1950 Professor P B Medawar and his English colleagues provided the basis for current experimentation upon ho

mologous skin transplants As a working schema Medawar (81) limited speculation about the causes of failure of skin homotransplants to three hypotheses blood incompatibility [4] geneticocellular differences [1–2] and acquired active immunity [6] Until recently the first two of these presumed causes of failure remained hypothetical It was known that transplants of skin between identical twins [5] were successful permanently but deservedly these have been termed autografts (78) Such grafts have been used to establish parenthood in cases in which there was doubt (62) With the increasing knowledge of the grouping of blood several cases have been reported of successful homografts of skin (83–84) where the donor and recipient had compatible blood groups [4] However knowledge of systems of blood groupings other than ABO has increased as has the knowledge of the Rh antigens Because it is now possible to divide human beings by the ABO Rh MNS P Kell Lutheran Lewis and Duffy systems with approximately 30,000 blood categories (85) which may be expanded to a million phenotypes it can be appreciated readily that this approach to the problem is far from exhausted It has been shown by Medawar (86–87) that leukocytes (but not red blood cells) and skin share some common antigens [10] He feels that there are at least seven independent antigens and that 127 skin groups [5] may exist Longmire (88) has postulated the existence of at least twenty three skin groups As yet this must stand as theory because the grouping of individuals by blood and by tissue is more complex than investigators had surmised

After a lifetime of work Loeb (89) advanced the theory that tissue incompatibility was due to organismal differentials [1] By this term now more historical than descriptive Loeb implied complex protein substances the chemistries of which are individual This is the theory of inherent or natural immunity It has been shown (90) that nucleoproteins are antigenic [9] and that proteins which are structurally different are also serologically different (91) It is also known that ribonucleic acid plays a role in the synthesis of the genes It is possible that genetic diversity may be associated with antigenic differences [2] This indicates that the closer the genetic relationship between the donor and the recipient the greater is the probability that homotransplantation will be successful (92) The genetic loci which are concerned with the susceptibility and resistance to homotransplants are H 1 H 2 and H-3 (histocompatibility 1 2 3) (93) Thus far however no differences have been demonstrated in the carbohydrate and protein metabolism (91) between auto and homografts of skin [19]

The now popular theory of acquired immunity [6] has received the greatest attention and enthusiasm from investigators. The keystone of this theory is the fact that the second set of homografts of skin from the same donor slough more rapidly than the first (92 94) Some investigators attribute this to the Arthus phenomenon in the rabbit [7].

The rate of loss of homografts of human skin transplanted subsequently in five successive homografts (95) is also accelerated [12] Dempster (96) has shown that the accelerated rate of loss of the second crop of homografts demonstrated first in the rabbit can be duplicated in the dog and therefore does not represent an Arthus phenomenon [7] Further he has shown that an initial kidney homotransplant will cause an accelerated rate of loss of subsequent homografts of skin Also homografts of skin will cause an accelerated rate of loss of a later homotransplant of the kidney [10] The time of survival of the secondary homograft is inversely proportional to the size or dosage of the transplant [13] From this and from experimental evidence it follows that it is better to use multiple donors in successive transplants to the same individual (97) than several crops from one donor [13] Medawar expressed the opinion that antibodies generated by homotransplants (9) prevent the completion of cellular mitosis [8]

Additional evidence which suggests that the loss of homotransplants may be due to acquired immunity is the constant finding of lymphoid cells (98–99) about the homograft and in increased numbers in the peripheral blood [11] The mechanism of immunity is understood poorly and the role of the lymphocyte and of the plasma cell in immunity still is a matter for speculation [11]. However there is evidence to support the contention that lymphoid cells may be the precursor for or the carrier of immune bodies (100) The first indirect evidence of the formation of antibodies against skin was shown by the treatment of free donor epidermal cells with the serum of an immunized recipient The donor epithelial cells were so altered as to be incapable of growth when reimplanted onto the donor [14]

In experimental animals some improvement can be achieved in the percentage of successful homografts of skin if the response of immunity is inhibited in the host Therapy by cortisone [18] and irradiation of the spleen [17] have been utilized to reduce the immune response Results reported by several investigators have varied Billingham and associates (101) have prolonged the lives of their homografts of skin by using cortisone locally in the rabbit while Cannon and Longmire (102) have increased by threefold the number of successful homografts by using cortisone in the chicken Others have found that cortisone or corticotrophin

does not prolong the survival time of homografts of skin in the human being (103–104)

*Attempts* have been made to reduce the ability of the host to respond immunologically by irradiation (105) of the entire body by splenectomy by splenic irradiation and by blocking the reticuloendothelial system by use of drugs [7] It is possible to achieve a high rate of permanently successful homotransplants of skin in the mouse by irradiation of the entire body and the results are enhanced (105) if the donor also is irradiated [14] It is difficult to counteract the reticuloendothelial system in its role in immunity for regeneration is rapid Temporary blockage of the reticuloendothelial function has been effected with trypan blue and with thorium dioxide (106) but convincing positive results are lacking

The conjoined placental circulation in synchorial dizygotic freemartin cattle may account for the success of homotransplants of skin between young freemartin cattle It is presumed that an acquired tolerance [20] exists between the dizygotic twins An attempt is being made to reproduce this tolerance experimentally in other animals (107)

Homotransplants have been altered to enhance their acceptance by the host [14 16] Freezing (95) has been used to alter the graft [15] Lyophilization or heating of a homotransplant has been used as an injection into the recipient before the homotransplantation of a second graft of skin Success of the homotransplants (108–110) has been increased by this means if the dosage is large but it has been decreased if the dosage is small [16]

## Comments

### [1] *Genetic-Cellular Hypothesis*

In one of his most recent (1953) papers, Loeb (111) stated

> Among various other substances functioning in a vertebrate organism there is present in all or almost all the tissues of an individual among the higher organisms a system of chemical substances which is identical in the organs and tissues of the same individual and which differs from that present in any other individual In this system there is one particular substance which characterizes an individual in contrast to a larger unit such as a species This chemical substance characteristic of an individual has been designated as his individuality differential The character of the individuality differential is determined by and representative of the set of nuclear genes of this individual
>
> A piece of normal ti ue or tumor when introduced into a host possessing a strange individuality differential calls forth antagonistic reactions on the part of various types of host cells the most characteristic of these is the reaction of the lymphocytes of the host which quantitatively indicates the degree of nearness or distance in the genetic relationship between host and transplant It is the host which reacts against the transplant Besides the lymphocytes the connective tissue cells and blood vessels of the host react against bearers of strange individuality differentials Furthermore immune substances which seem to be carried by the blood serum of the host and in all probability also preformed substances present in the blood serum may act against the strange organismal differentials (111)

The above paragraphs summarize the essence of Loeb s genetic-cellular hypothesis. Thus it can be seen contrary to the derogatory remarks of some researchers in recent years that Loeb definitely recognizes the probability of a systemic reaction against skin homografts as well as the presence of 'immune substances.' Although his hypothesis, when first proposed, stressed a primarily local cellular or local lymphocytic response as the chief agent bringing about destruction of incompatible tissues this emphasis has changed with the passing of time and with undoubtedly considerable hindsight. Loeb like everyone else interested in transplantation advances could not help being aware of the meticulous studies of Medawar (8 9) and his group (10 30) which demonstrate the systemic role played by actively acquired immune antibodies in the destruction of skin homografts.

But it is still historically more accurate to emphasize that Loeb in his 1953 review, still did not think primarily in terms of immune mechanisms. His role in the current history of homotransplantation has been largely to stress again and again the ever present genetic factors that determine the severity of the host s reaction against homografted tissues. Throughout all his research work, however Loeb was perhaps too greatly impressed with the lymphocytic infiltration that occurs in the region of many tissue homografts (see [11]) The chief criticism of Loeb s theory is that it devotes too much attention to the importance of histology e.g the local cellular reaction, and not enough to the systemic immune mechanisms involved.

The eventual destruction of homografts and heterografts alike Loeb considered was the result of homotoxins and 'heterotoxins.

These cytotoxins were essentially the product of the action of the graft's "individuality differentials" upon the host. Accordingly Loeb believed that the graft had a direct toxic effect upon the host and that immunity factors if present were of secondary or minor importance. Some workers in subsequent research have suggested that Loeb's terminology be paraphrased with the word antigen substituted for "individuality differential. The differentials therefore, become "antigens" of the grafted tissue which are specific for the particular graft and host concerned.

Burnet and Fenner (112) who did much in their review to bridge the gap between supporters of Loeb's theory and the more recent advocates of Gibson and Medawar's acquired immunity theory (92) wrote "... it has long been realized that the basis of the differences between host and donor tissue was genetic." And in this regard a lifetime of work by Loeb has at least not been unrecognized In his exhaustive studies beginning in 1897 (113) with the transplantation of white and of pigmented guinea pig skin, Loeb repeatedly emphasized his strong belief in the inheritance of factors that determine the ultimate behavior of normal and neoplastic tissue homografts. His theories have been borne out by studies of skin homografting both in animals and in man

The majority of research on skin homografts and on the biologic laws that govern their behavior however has been conducted in animals. Excellent experiments have been performed in excellent laboratories, with assiduous use of adequate "control material Equivalent "control" material in human experiments however has been relatively unknown in the medical literature until very recently (114 115)

For many years surgeons have preferred to use family members as donors of skin to the severely burned patient requiring homografting. It has seemed to be merely a matter of common sense that skin grafts from a close relative would be tolerated better by the host than those supplied by non-related individuals Nevertheless when skin supplied by non-related donors has been used, it too has served fairly well as a temporary burn dressing before it undergoes typical homograft rejection and slough Loeb's emphasis on the longer survival time of tissues taken from closely related familial donors (syngenesiotransplantation) in comparison to the survival time of tissues from non-related non familial donors (homoiotransplantation) led the present author to conduct a series of human homograft experiments which have covered the span of years from 1951 to 1957 The results seem to shed new light on the advantage of using as donor material skin from members of the same or closely related families when homografting (114)

Results from transplanting full thickness circular skin homografts of a uniform diameter to the anterior thigh, volar forearm, or medial arm surfaces of human volunteers whose esthetic or cosmetic preferences determined the site indicate the following preliminary conclusions (114) 1) Full thickness reciprocal skin homografts transplanted under controlled conditions in adult non-related human volunteers chosen at random survive for only 7 to 9 days postoperatively 2) Full thickness reciprocal skin homografts transplanted under controlled conditions in closely related adult individuals (e.g., dizygotic twins) survive for periods ranging from 19 to 29 days postoperatively 3) Full thickness reciprocal skin homografts survive permanently when transplanted under controlled conditions in monozygotic (identical) twins 4) When skin homografts are obtained from a closely related human donor (syngenesiotransplants) survival in the recipient under controlled conditions is longer than that of skin homografts obtained from non-related donors. 5) In treating the severely burned patient therefore when skin homografting is indicated donor graft material should preferably be taken from closely related members of the patient's own family e.g mother sisters brothers (114)

Peer (115) carrying controlled reciprocal homografting one step further in a study of acquired tolerance (see [20]) has demonstrated that a mother is apparently the best source of routine donor material for skin homografting when both parents and grandparents are available as donors In a study of full thickness skin homografts taken from fathers and mothers and placed upon their 2 to 3-month-old infants the longest survival times were shown by skin grafts donated by mothers. Such grafts from mother to infant were rejected as late as 20 days 21 days 86 days and 200 days postoperatively A rather surprising finding, and one that was not anticipated was the still longer survival time of the infant's skin transplanted to the mother (Peer placed his reciprocal skin "switch homograft" behind the ear of both parent and child) The

infant grafts placed behind mothers' ears were still viable at the time of Peer's report (115). The survival times were thus to that date 56, 140, 200, 321 days postoperatively, and none of the grafts has been rejected as yet. Peer found it difficult to explain this longer survival of infant skin homografts on maternal hosts. His findings are especially interesting since the work of Loeb and others has demonstrated that host reactions are generally more severe "... when tissues of the ... offspring were transplanted into the parents than when tissues of the parents were transplanted into the offspring" (111).

Peer suggested that the longer survival of skin homografts from mother to infant and from infant to mother compared to the survival of homografts from father to infant could possibly be attributed to a partial "tolerance" between mother and child resulting from an intermingling of fetal and maternal circulations in certain instances (see [8] and [20]).

## [2] Genetic Diversity and Antigenic Differences

In 1938 Gorer (116) demonstrated that dominant genes are responsible for antigenic differences. Antigens are usually foreign proteins. Scothorne and Tough (91) in histochemical studies of skin autografts and homografts concluded that the host's antibody activity in homografting might be directed against a possible antigenic ribonucleic acid in the cytoplasm of homografted cells. Cytoplasmic ribonucleic acid is usually found combined with a protein as ribonucleoprotein, thereby satisfying the usual requirement that antigens are protein in nature. Caspersson (117) observed that ribonucleic acid is probably involved in the synthesis of genes. Thus, from an antigenic standpoint "... if structural differences do exist between the epidermal ribonucleic acids of different individuals, it may be assumed that the ribonucleic acid confers structural and therefore serological specificity upon the protein molecules with which it is combined as ribonucleoprotein" (91).

In essence, the work of Gorer, Snell, Scothorne and Tough (91, 116, 118–120) and others reveals that the host probably responds to antigens found in donor tissues that are absent in its own tissues. And the presence of antigens in the host that are lacking in donor tissue seems to be of little or no importance whatsoever as far as the host's response to the donor graft is concerned.

Since antigenic differences are determined by dominant genes (116), it would perhaps be better to say that the host responds to dominant genes found in the donor tissue that are absent from its own tissues. Snell (118) in 1948 called these dominant genes, "histocompatibility genes." Although he studied them primarily in experiments with tumor transplants, Snell believes that the same histocompatibility genes "... that determine susceptibility and resistance to tumor transplants probably also determine susceptibility and resistance to normal tissue transplants ..." (119). This is consonant with Gorer's generalization (1942): "... The laws governing the transplantation of normal and neoplastic tissues are essentially the same ..." (120).

Histocompatibility genes may be either "strong" or "weak." Thus the histocompatibility gene or genetic locus known as $H_2$ is a "strong" locus. A genetic "locus" refers to the site or position of the particular gene involved on the long axis or span of a chromosome. Differences between a tumor and a host at the "strong" $H_2$ locus will impede or prevent the growth of almost all but the most virulent types of tumor tissue (119). A "weak" histocompatibility gene is one "... such that a tumor carrying it will often grow in a strain lacking it." Thus, histocompatibility genes determine not only the susceptibility to tumorous and survival of normal tissue homografts but the resistance to tumorous and rejection of normal tissue homografts as well (119).

A slight familiarity with the geneticist's terms will undoubtedly help to clarify to those involved in normal tissue transplantation the interrelationships which are continuously being revealed between the two fields. The fact and the currency of these relationships are well illustrated by the titles in a recent issue of the *Transplantation Bulletin* (121): *The detection of antigenic differences attributable to the Y chromosome; Histocompatibility genetics of scale transplantation; Apparent loss of specific iso-antigens in heteroploid transplanted tumor cells; Mechanism of induced change in transplantation specificity of a mouse tumor passed through hybrid hosts; The cytotoxic activity of isoantibodies in mice; Response of rabbits to defined antigens following neonatal injections; Renal homotransplantation in goats.* These and other recent papers meet the clinician's need to grasp concepts and terminology by means other than exhaustive, time-consuming

study For example, allele becomes a manageable term to the clinician who reads a review such as *The genetics of transplantation* (119) even though it deals primarily with tumor transplantation in mice, a laboratory excursion far removed from the daily traffic of the clinic In this paper Snell explains

"We have so far spoken of the gene H 2 as if it were a single entity Actually as is commonly the case with genes in all plants and animals it exists in several alternative forms These alternative forms are called alleles."

The term is thus introduced and defined and its pertinence emerges when the clinician learns for example that the blood group subfactors, C D and E are alleles of the Rh genes in man.

[3] *Twins*

Contrary to a rather widespread belief "identical" blood group factors (including the C D and E subgroupings just mentioned) are not characteristic only of "identical or monozygotic twins. Osborne (122) recently demonstrated that among some sex twins having the same blood group factors approximately 15 to 18 per cent may be dizygotic or "non-identical Neither blood grouping therefore nor fingerprinting can any longer be used as an undisputed criterion for the diagnosis of monozygosity or identical twinning It is in such cases where the diagnosis of monozygosity or dizygosity is uncertain that skin homografting has recently proved a more accurate guide (114)

Whereas skin homografts involving two human individuals of ordinary genetic diversity are almost invariably rejected by the host individual homografts exchanged between monozygotic twins survive permanently (114) Skin homografts exchanged between dizygotic (human) twins however are ultimately rejected in periods ranging from the eighteenth to the twenty ninth day postoperatively Skin homografts exchanged between *dizygotic twin cattle* on the other hand frequently survive indefinitely (107) and behave in all respects like autografts This is in contrast to the rejection of homografts exchanged between ordinary cattle siblings or between dizygotic twin sheep (123) The contrast between cattle and sheep in this respect has been attributed to the interchange of fetal blood in cattle twins as is evidenced by the occurrence of freemartins in cattle Furthermore in such cattle each member of a dizygotic twin pair usually has two types of red blood cells a heterogeneity suggestive of an actual transplantation of blood forming tissues by way of the fetal vascular anastomoses Such animals are spoken of as *erythrocyte chimeras* (107) At least one such chimera has been described in man (121)

Skin homografting has been used in several interesting approaches somewhat in the manner of medical detective work McIndoe and Franceschetti (82) employed skin homografts in a remarkably simple yet ingenious manner to identify identical twins from among three children who had apparently been mistakenly exchanged soon after birth in the hospital nursery where all three had been born McIndoe and Franceschetti described this medicolegal problem in the following interesting excerpt In 1947 the parents of 6-year-old twins (Victor and Pierre J ) became aware of the existence of another small boy (Eric V ) who presented a striking resemblance to one of their own children Believing first that it was *simple coincidence they were surprised to learn* that the other child was born the same night, and in the same clinic as their own During a parade in which the similarly dressed children were participating the father was shocked by the resemblance and decided to contact the authorities in order to learn whether or not a substitution of one *of his twins could have taken place* (82)

Full thickness skin homografts transplanted between the two boys who had been raised together as twin brothers necrosed and sloughed off whereas skin homografts between Victor and Eric the true twins survived permanently This story unique both from the scientific and emotional point of view had an interesting epilogue in accordance with the conclusions of the investigation the authorities ordered the exchange of the substituted children The adaptation of the children to their new environment is as personal inquiries have confirmed satisfactory " (82)

Rogers and his associates (114 125 126) had *the opportunity to study three pairs of twins* in whom monozygosity or dizygosity was not clearly established despite exhaustive genetic tests Since it is generally believed that nonidentical twins are no more closely related than ordinary brothers and sisters, it was expected that skin homografts would ultimately slough if transplanted between dizygotic or non identical twins With this belief as a guide skin homografting was employed to clarify the role of heredity vs acquired congenital

factors in the etiology of Mongolism and mental retardation One member of each pair of twins was mentally retarded In one case an 11 year old boy was a mongoloid his twin was normal (fig 55) Was heredity a factor in the cause of this twin's mongolism? In another case a 13-year old girl showed severe mental and physical retardation her twin was also normal Was a birth deformity responsible for the affected twin's deformities? In the third set of twins one 10-year-old girl was a mongoloid her twin was normal. A diagnosis of monozygosity in any of these three sets of twins would make less plausible the theory of heredity as an etiologic factor considering the normalcy of the other twin

Full thickness, circular skin homografts were transplanted in corresponding defects made on the volar surface of the forearms of each set of twins Monozygosity or identical twinning was established in the 13-year-old girl twins by permanent survival of their cross transplanted homografts (fig 56) In the other two sets of twins, dizygosity was diagnosed by rejection of the skin homografts at varying periods, ranging between the nineteenth and twenty ninth postoperative day (fig 57)

Use of skin homografts therefore can be employed to diagnose conclusively human monozygosity or dizygosity for purposes of determining the etiology of hereditary and congenitally acquired abnormalities and in certain cases presenting medicolegal quandaries



Fig 55 Dizygotic twins in whom reciprocal skin homografts established the diagnosis of dizygosity The upper frames show the mongoloid twin and the lower frames show the normal twin (From B O Rogers The genetics of skin homo transplantation in the human Ann New York Acad Sc 64:741 1957 )

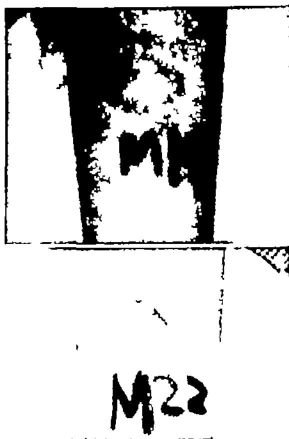



Fig 56 Normal healthy appearance of reciprocal skin homograft donated by a monozygotic twin and transplanted to the volar forearm surface of the other twin on the nineteenth postoperative day (*above*) and the twenty-second post operative day (*below*) The appearance of the homograft noted in the lower frame is still the same today several years after the homografting procedure thus suggesting permanent survival (From B O Rogers The genetics of skin homo transplantation in the human Ann New York Acad Sc 64 741 1957 )

### [4] *Blood Group Compatibility and Homograft Behavior*

'Cells such as erythrocytes which have no power to elicit transplantation immunity are incapable of causing tolerance of tissue homografts (10) With this statement based upon exhaustive quantitative studies on tissue transplantation immunity Billingham, Brent and Medawar reemphasized the earlier contentions of Medawar (87) that blood group compatibility or blood group antigen compatibility has little influence upon the ultimate outcome of the survival or sloughing of most skin homografts

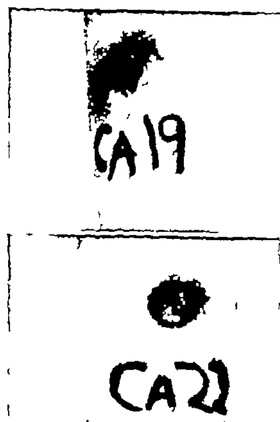



Fig 67 Skin homograft donated by a dizygotic human twin and transplanted to the volar forearm surface of the other twin on the nineteenth post operative day (*above*) and the twenty-second postoperative day (*below*) In the upper frame note the onset of induration confined only to the homograft In the lower frame induration hemorrhage and ulceration indicative of "homograft rejection suggested that these twins were not identical and thus a diagnosis of dizygosity was established. (From B O Rogers The genetics of skin homotransplantation in the human Ann New York Acad Sc 64: 741 1957 )

It must be pointed out however that there is another school of thought which bears upon this subject Gorer for example described a human case that seemingly demonstrated some antigens shared by human skin and red blood cells (127) A severely burned girl received skin homografts from her father no blood transfusions were administered from father to daughter Both the patient and father were of blood group A Rh negative The patient s serum contained no demonstrable antibodies against the father s erythrocytes prior to the grafting procedure Following the sloughing of the homografts donated by the father however the serum of the patient showed a positive antiglobulin reaction with the father s erythrocytes The conclusion that may be drawn from this case is that human skin and erythrocytes probably share some antigens in common. The father s homograft probably introduced individual-specific skin antigens into the daughter stimulating the formation of individual-specific antibodies

In previous genetic experiments with mice Gorer also demonstrated that an incompatibility of antigens found in the erythrocytes of two mice was associated with an incompatibility and rapid destruction of other tissues primarily tumors homografted from one mouse to the other In some experiments compatibility of the red blood cell or erythrocytic antigens guaranteed the persistence or survival of other tissues homografted between the two mice (127) Barrett and Hansen (128) demonstrated that prior injections of washed red blood cells and even red cell stromata or portions of the red cell membrane under proper conditions, would induce a marked degree of resistance or immunity against subsequent tumor homografts from the same donors

In the light of these controversial and contradictory findings it is a matter of speculation whether extremely delicate blood typing techniques as yet undeveloped might possibly result in the identification of new blood subgroups that might conceivably play some rôle in skin homografting Simonsen (129) suggested that one must aim at thorough congruity of the blood group combinations in donor and recipient. This is a complicated task which, however is not impossible to solve If the result proves negative we still do not know whether this is because blood groups play no part in skin homografting or whether there are still undiscovered blood groups of equally great or even greater importance than those already known

## [5] *Skin Antigens and "Skin Groups"*

In Medawar s pioneering account the subject of "skin groups" analogous to blood groups was discussed (81)

> A comparatively small number of distinct antigenic factors offer by their combination two wise a large number of distinct groups Thus 00 blood genotypes are determined by the independent assortment of the A1 A2 B-0 with the M N and Rh group characters alone A review of the surgical evidence goes to show that epidermal groups if they exist could hardly number less than 100

The idea that comparatively few genes may

control tissue compatibility is supported by the natural history of grafting phenomena. Homoplastic skin interchange is possible between adult Urodela (130) though not between adult Anura (131) yet it can hardly be denied that individual Urodeles are genetically distinct. Skin incompatibility is well established in evolution at the level of the Reptilia (132) and there is suggestive evidence that some species of reptiles show a distinct segregation of isohaemagglutinogens into groups (133). It is possible then that blood polymorphism and tissue incompatibility evolve hand in hand and that the same type of genetic differentiation underlies them both. Unfortunately an attempt to work out the distribution of say skin groups in human beings would need an *in vitro* test for compatibility that has yet to be devised.

Attempts to "type" skin in the fourteen years subsequent to Medawar's discussion (81) have been unsuccessful (134) to date. Since a skin "type" or "group" would be determined by its genetic composition it is again important to emphasize that the host responds to genotypes or histocompatibility genes in the donor's tissue that are absent in its own tissues. Various estimates have been made of the least possible number of genotypes or genetic antigens acting in any reciprocal cross skin homografting between two individuals. A recent study by Barnes and Krohn (135) has shown for example that in certain mice strains not less than fifteen independent genes or separate genetic antigens are controlling the fate of any skin homograft transplanted between two mice.

It must be stressed that this is the *minimal* number of antigens that come into play in this animal species. A human study in which 71 unrelated donors provided skin for homografting one severely burned patient led Longmire and associates (88) to conclude that, if skin groups do exist, it is probably unlikely that there are less than 23 separate ones determining the fate of human skin homografts.

## [6] *Actively Acquired Immunity in Skin Homografting*

Research prior to and subsequent to Medawar's basic studies in skin homografting (8 0 81 92) tends to confirm the hypothesis of acquired immunity as an explanation of the host's rejection of a skin homograft.

Clarification of the exact nature of this immunologic reaction is one of the primary goals of homograft research. The theory of acquired immunity can be traced back to the work of Schoene who in 1912, suggested its rôle in tumor transplantation and thus by analogy, in the grafting of normal tissues (136–137). Schoene pretreated animals with a homologous "brei" or mash of liver cells and then homografted them at a subsequent date. In the region of the homograft a greatly increased and intensified cellular response was observed which he attributed to an immunity reaction.

The modern theory of acquired immunity was apparently first formulated by Thomas Gibson (92) a Scottish plastic surgeon, in a pioneering paper entitled *The Fate of Skin Homografts in Man*, published in 1943. On the basis of previous unpublished experiments in which "first-set" and "second-set" skin homografts were transplanted from the same donors to the same respective human host the more rapid rejection of second-set grafts suggested to Gibson the important role of an actively acquired immunity in rejection of skin homografts. Gibson's co-author of this paper was Medawar from whom the field of transplantation was soon to hear a great deal. In fact Medawar can be credited with popularizing and further clarifying Gibson's hypothesis in the years that followed.

When skin is transplanted as a homograft its vessels become filled with blood from the host. The blood is seen to circulate freely; the gross and microscopic appearance of the homograft is comparable to that of an autograft. After a period of a little more than a week however the flow of blood in the vessels of the homograft ceases, the vessels become thrombosed and the graft is rejected (73).

Acquired immunity is suggested by the time factor involved in these phenomena. A lapse of five to seven days usually occurs before evidences of rejection such as vascular thrombosis and necrosis, occur. This period is suggestive of the average time usually required for the body to build up effective antibody levels against foreign antigens (138).

The "second set phenomenon" (8) seems to be another factor of immunologic significance. A second set of skin homografts (fig. 58) taken from the same donor and transplanted to the same host sloughs more rapidly than the first set of homografts (8, 114). The shorter survival time

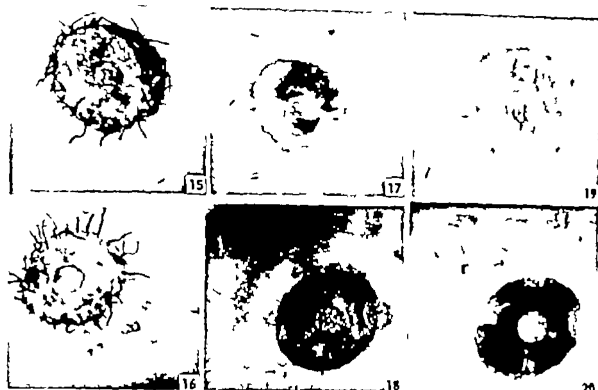



FIG 58 Comparison of behavior and survival of first-set and second set skin homografts in the human host

*First set.* 15 Fifth postoperative day initial take The graft is viable 17 Ninth postoperative day onset of hemorrhage and vascular thromboses Some portions of the graft are still viable 19 Twelfth postoperative day the epithelium is desquamating exposing the raw dermal bed

*Second set.* 16 *Third postoperative day despite the initial take the graft is severely thrombosed* and hemorrhagic 18 Tenth postoperative day the graft is dry and gangrenous The epithelium has sloughed 20 Twelfth postoperative day the graft is completely and intensely gangrenous (From B O Rogers The genetics of skin homotransplantation in the human Ann. New York Acad Sc 64: 741 1957 )

of second-set grafts may be attributed to an acquired immunity reaction, *i.e.*, immunity previously developed in the hosts against the presence of first-set grafts.

Studies of second-set bone homografts reveal that the inflammatory reaction in the region of these grafts is more intense than that observed around the first-set homografts (139) This constitutes but one of many additional circumstances which indicate that the homografted individual acquires a state of immunity

### [7] *Type of Immunity Involved in Homografting*

Investigative attempts to identify classify or pigeon hole the acquired immunity reaction involved in homografting have been concerned recently with a comparison of the homograft reaction with the Arthus phenomenon the Schwartzman phenomenon delayed hypersensitivity of the tuberculin type etc

Lawrence (140) pointed out that choices between an Arthus type of hypersensitive reaction and the delayed tuberculin type as an explanation of the rejection of homografts cannot be conclusive These two types of hypersensitive reactions do not necessarily contradict each other from the immunologic standpoint. Immunologists are becoming increasingly aware that both types of hypersensitivity reactions may in reality represent variations of similar if not identical, immunologic mechanisms There are data which suggest that the tuberculin type of hypersensitive reaction is merely an incomplete or early phase of the Arthus reaction

For many years the failure to demonstrate serum antibodies in the response to homograft antigen led workers to believe that cellular antibodies play the most important role in neutralizing homograft antigen Interestingly the delayed tuberculin reaction is strictly a cellular type of phenomenon (138) The successful transfer of

this immunity from one individual to another depends upon the use of cells of an exudative nature (141) *e.g.*, lymphocytes or lymph node creams. In a sloughing homograft lymphocytes are abundantly present in and about the graft (89)

It may also be noted that passive transfer of immunity against the homograft can occur if lymph node tissue is transplanted from immune animals to non-immune animals (142 143) Attempts at passive transfer by serum have not, as yet, been successful (142, 144) Although this does not eliminate the importance of serum antibodies, it does suggest that serum or circulating antibodies play a lesser role in the usual reaction to homografts than cellular or leukocytic antibodies. Heterologously produced serum antibodies in large quantities may actually enhance the growth and increase the survival time of some homografts (*e.g.*, tumor) rather than hasten their rejection (145) Billingham and Brent (144) concluded that their inability to transfer immunity against skin homografts by passive immunization techniques was in agreement with the thesis that the agent of skin homograft destruction is not present in the serum of immune animals and that transplantation immunity is transferable with cells only

Lawrence emphasized (140) that a comparison of the response to the homograft with that of delayed tuberculin hypersensitivity reveals fairly consistent similarity 1) induction of sensitivity in both responses requires intact cells (or bacteria) 2) the latent period before the reaction is roughly identical in both responses 3) the specificity involved is specific only for the original donor cells or donor bacteria (tubercle bacilli) 4) the presence of any measurable serum antibody is variable in both responses 5) in neither response has there been any demonstrable parallelism between the degree of actual hypersensitivity that occurs and the amount of serum antibody and finally 6) attempts to transfer the sensitivity in both responses by way of the serum have been unsuccessful whereas the sensitivity is successfully transferred in both responses when immune cells are used

### [8] *Effects of Immune Antibodies on Skin Homografts*

In neutralization of homograft antigens the antigen antibody reaction taking place locally at the site of the homograft is understandably of a severely destructive nature It must be in order for the host to rid itself of the offensive foreign body or foreign proteins in the graft Medawar (146) demonstrated the complete suppression of mitotic activity in epidermal cells of skin homografts transplanted to a rabbit previously immunized by a first-set homograft He concluded that the immune state called forth by grafting foreign homologous skin is directed against the reproductive mechanism of the transplanted cells which is evidently put out of order rather than against the cells 'vegetative activities which are not immediately affected The latter is a reference to the fact that although the epidermal cells of these grafts in immune animals suffer a complete suppression of mitosis the grafts themselves may survive in a vegetative condition for a period almost always greater than 4 days though usually less than 8 (146)

Coupled with the effect of antigen-antibody neutralization on cellular mitosis the homograft reaction is also typified by a violent inflammatory response (see [11]) and a vascular breakdown in first-set homografts. Medawar (146) attributes these events to sensitization' They are quite similar to those pathologic changes seen in other hypersensitive reactions. No laboratory studies have been reported since Medawar s basic experiments (8 9) which add essentially to the wealth of concise evidence found in his comparative histologic description of the 'homograft reaction

The evolution of autografts falls into a period of primary healing and vascularization a period of generalized hyperplasia in which all the cellular elements of the grafts participate and a period of partially retrograde differentiation during which the grafts return towards the condition of normal skin

Homografts undergo normal primary healing in a latent period during which they provoke no specific reaction from their recipients At some time thereafter they are invariably destroyed

The evolution of homografts embraces the period of generalized hyperplasia of autografts but does not usually extend into the period of differentiation New hairs do not mature and pierce the graft roof

The phenomena of acute inflammation are superimposed upon the otherwise autograft like behaviour of homografts in the period of hyperplasia The inflammatory process includes vascular and lymphatic proliferation a

massive invasion of the grafts by lymphocytes and monocytes of native origin through the walls of the vessels within them edema of such severity that the graft bed may be distended by tracts of free fibrinous matter and a general mobilization of mesenchyme cells

Inflammation reaches its peak and passes into necrosis with the stagnation and obliteration of the vascular system of the graft and the death of every cellular element within it Homografts are then invaded anew by capillary vessels from the graft bed lymphocytes and monocytes passing through their walls establish a secondary population of native cells within them The intensity of the inflammation and its rate of development vary inversely with the time of survival of foreign skin epithelium

Disengagement and breakdown of the foreign epithelium begins in that which has spread from the graft and extends thereafter to epithelium of the graft center to the accompaniment of a variety of non-specific pathological changes in the cells (8)

Taylor and Lehrfeld (147) observing the destruction of skin homografts with a stereoscopic dissecting microscope concluded that the chain of events in the rejection of skin homografts begins with an attack on the endothelium of the homograft capillaries by the destructive agents liberated in the antigen-antibody reaction Thus the vascular system is involved prior to any degenerative and necrotic changes These authors suggest that the 'survival time' of a skin homograft be defined as that period which elapses from the time of transplantation to the first signs of circulatory impairment within the homograft The progressive signs of homograft rejection thus become 1) stasis or stagnation of blood flow 2) vascular thromboses 3) increased vascular permeability 4) rupture of the vessel walls, and finally 5) spillage or diffusion of erythrocytes into the perivascular tissue spaces of the graft (148) Taylor and Lehrfeld believe that the subsequent necrosis and degeneration of the homograft is a direct result of lack of circulation (147)

## [9] *Type of Antigens Involved in Skin Homografting*

Medawar (149) recently reported that the antigens probably responsible for homograft reactions are confined to the nuclei or nuclear fraction of cells and they either are or are intimately associated with chromosomal nucleoprotein Rogers (78) reviewing in 1950 the problems of skin homografting wrote If it is an enzymological nucleoprotein reaction is this a direct reflection perhaps of the complex nucleoproteins we know to exist in the nuclei and therefore the chromosomal-gene elements of every cell? Could there be a direct nucleoprotein antagonism between donor and host cells analogous to the neutralizing antigen-antibody reaction? *It should be stressed however that current research has not yet ruled out the possibility that homograft immunity antigens might also be found in the non nuclear portions of homografted cells*

If Medawar's findings prove consistent Gorer's dictum (116) might be revised by substituting "chromosomal nucleoprotein" for gene It would then be fitting to say that antigenic differences between host and donor are determined by dominant genes, the host responding to chromosomal nucleoproteins in the donor tissue that are absent from its own tissues. Medawar suggested that if chromosomal DNA (desoxyribonucleic acid) is the determining antigen in skin homograft reactions then it is probably a nucleoprotein of low molecular weight (149)

## [10] *"Individual specific" Shared Antigens in Homografting*

Circumstantial evidence has indicated the existence of individual-specific cellular antigens common to different cells or tissues of the *same* animal or individual These antigens although specific for the individual animal are also "non-specific" in the sense that they are not unique to one cell type Injection of leukocytes containing such antigens stimulates antibody production in the host animal this antibody reacts not only against the injected homologous leukocytes but also against subsequent skin grafts from the same donor (87) These results signify that some antigens are shared in common by both leukocytes and skin Tumor and normal adult tissue homografts may be rejected because they contain a whole spectrum of these antigens rather than a single specific antigen which alone is the responsible factor (see [5])

The cells, tissues and organs of an individual may contain a constellation of antigens probably similar to those found in the human rhesus blood system and perhaps as varied and complex (127) Genetic experiments with mice have indicated that the incompatibility of antigen found in the erythrocytes of two animals re-

sulted in the incompatibility and rapid destruction of other tissues homotransplanted from one animal to the other In some experiments compatibility of the erythrocytic antigens guaranteed survival of other tissues homografted between the pairs of animals (127)

In the case history described under *Blood Group Compatibility and Homograft Behavior* (see [4]) it was demonstrated that human skin and erythrocytes probably have some antigens in common. In the case referred to individual specific antibodies incited in the host by individual-specific antigens of a skin homograft neutralized not only the foreign skin antigens but also those antigens of the donor red blood cells which were native also to the related host (127) Other studies reveal that skin and cornea share common antigens, since a more rapid destruction of second-set corneal homografts or first-set skin homografts from the same donor occurs if both are preceded by a corneal homograft transplanted into the abdominal wall of the host (150) In another experimental study antigens shared by skin and kidney homografts have been demonstrated and antigens shared by kidney and erythrocyte homografts have also been detected (151)

### [11] *Role of Leukocytes in Skin Homografting*

The breakdown of skin homografts is almost always associated with an infiltration of lymphocytes into the graft Loeb (89) and others believed that the invading lymphocytes were at least in part responsible for the destruction Darcy (152) studying the behavior of lymphocytes and plasma cells in first and second-set homografts, concluded that a more likely hypothesis for a causal relationship between the cellular invasion and graft destruction is that the cells liberate specific antibody at the site of the graft It is possible to reconcile such a mechanism with the systemic effect of postulating that lymphocytes and plasma cells, specifically modified through the action of the graft antigen on the hemopoietic centers are liberated into the circulation and carried to the graft Alternatively a slight reaction of circulating antibody with the graft might attract the cells. More recent laboratory evidence supports the concepts of Darcy He further stated

The invading cells were found to include besides small lymphocytes a large number of cells staining strongly with pyronin Some of the latter were characteristic mature plasma cells the rest were according to the evidence probably immature ones The immature forms were largely lymphocytic in appearance but some had reticular cell characteristics maturation occurred at the graft site It appeared likely that a fate of the non pyronin staining lymphocytes was to become plasma cells but others underwent destruction at the time of failure of the graft s blood vessels

Scothorne (153) described the formation of many large lymphoid cells in the cortex of regional lymph nodes draining an area where skin homografts have been transplanted He suggested that the large lymphoid cell is a member of the same family of reticuloendothelial cells producing antibodies against bacterial and other types of antigens

Ehrlich and Harris (154) were among the first to propose that the actual site of antibody formation lay in lymphocytes More recent investigations by Scandinavian immunologists have placed greater emphasis on the plasma cell (fig 59) which is certainly closely related to the lymphocyte. Cushing and Campbell (155) concluded in their recent immunologic review

Regardless of the specific cell type which is involved in antibody formation it seems evident

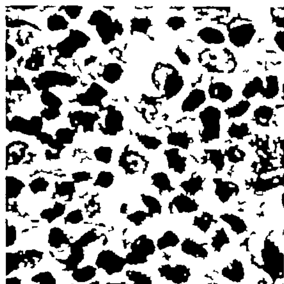


Fig 59 Photomicrograph showing plasma cell invasion in an area of homograft rejection (From R L Varco L D MacLean J B Aust and R A Good Agammaglobulinemia an approach to homovital transplantation Ann Surg 142 334 1955 )

that all types are closely related Whether they are lymphoid elements or large phagocytic macrophages they are all derived from the same basic reticular cells which have the potential to develop all these different cells (including plasma cells)

The eosinophil has also been directly implicated in the rejection of skin homografts Rogers and his associates (156) have demonstrated that circulatory eosinophilia is pronounced during the *rejection of skin homografts in the human and* levels may rise to 4000 per cu mm Eosinophils have also been observed in the host tissues lying *beneath around and even in the sloughing skin homograft (70 156 157) These cells have been* regarded for many years as direct evidence of an allergic response (138) In Rogers study circulatory eosinophilia disappeared 30 to 40 days post operatively when sloughing of the skin homograft was completed Cooke (138) concluded from these studies that the dermal homograft was clearly the stimulus for eosinophilia.

In a discussion of his findings, Rogers (158) suggested in 1953

> We strongly favor the hypothesis that the eosinophilia is tell tale evidence of an 'ill defined allergic response taking place in the tissues which is somewhat similar to the response seen in skin sensitivity reactions with ulceration as described by allergists or as seen in the tuberculin reaction

In a recent summary of the physiology of eosinophils Speirs (159) stated

> 'The relation of the eosinophile to the antigen antibody reaction warrants much more study It has been clearly demonstrated that adrenalectomy causes a tremendous local increase in the number of eosinophiles responding to antigen injections in sensitized mice

### (12) Sequence of Skin Homografting and "Dosage"

Although Medawar has conclusively shown the existence of a dosage phenomenon in animals (the greater the amount of skin homografted the shorter the survival time) there is no evidence as yet to suggest that dosage plays any role in man There are as many clinical cases of large dermatome grafts persisting for 6 weeks or more as there are cases of only a few punch grafts sloughing in 2 weeks and vice versa Then is apparently neither a direct nor an inverse relationship between skin dosage and survival time of the grafts In human studies full thickness skin homografts experimentally applied show a fairly consistent survival time of 7 to 9 days with a mean time of 8 09 days whether their diameters are 11 mm (73) or 35 mm. (114) Survival time of skin homografts in man is more often controlled by the genetic relationship between donor and host (114) and/or the tendency of the burned individual *who is homografted to show a greater excretion* of urinary corticosteroids and higher blood levels of corticoids (161) The natural stress response of the adrenals is surprisingly marked in critically burned patients (162)

The increased corticosteroid activity in burned individuals is an analogous situation to the studies of Billingham Krohn and Medawar (101 163) and of numerous more recent investigators who have amply demonstrated that skin homograft survival time can be significantly prolonged by administration of cortisone or other corticosteroids

As far as the influence of a 'time sequence" in skin homografting is concerned it has been demonstrated that second-set grafts in man survive for a shorter period of time than do first-set grafts (114) Third set corneal grafts transplanted to the rabbit s thoracic wall survived for the same period of time as did second set grafts (164) Rapaport and Converse (165) observed similar results in repeated skin homografts involving the same donor and host They *noted no significant difference in the survival* times of second third and fourth-set graft each graft surviving for a period of only 4 to 5 days compared to the 9- to 10-day survival of *first-set grafts Thus in their controlled study* third and fourth-set grafts were rejected in an accelerated manner typical of second-set graft Interestingly a skin homograft from the same donor applied to the recipient *80 days after* rejection of the fourth-set homograft behaved as a first-set homograft (165) They concluded that in man a specific sensitivity to skin homografts will persist only as long as the [homograft] antigen or its derivatives are present in the host

### (13) Multiple Donors and Survival Time of Skin Homografts

In regard to animals where the dosage phenomenon applies Dempster and Lennox (67)

speculated that a number of small skin homografts from several donors would survive longer than grafts of a similar total size obtained from a single donor. Their experiments in rabbits confirmed this hypothesis. As a result, they recommended for extensively burned patients the use of small homografts procured from many donors rather than the use of a single donor. Mowlem (160) obtained satisfactory results with burned patients by alternating autografts with homografts from multiple donors.

> As the grafts from some donors may be expected to survive longer than others it would probably be an advantage to mix them in as small patterned a mosaic as possible so that the areas to be covered by advancing host epidermis at any one time will be as small as possible (97).

Although the clinical procedure of using multiple donors has not been tested in any controlled study of burned patients to date, the reviews by Rogers (114) and Peer (115) would suggest a further refinement: because skin homografts in man survive longer when obtained from the patient's immediate family and close relatives, it might be advantageous to alternate autografts with homografts obtained from many members of the patient's *family*. This procedure assumes of course that the dosage phenomenon is also applicable to man, an assumption not yet demonstrated clinically (see [12]). Even if it were not applicable, however, a small mosaic pattern of homografts from many donors alternating with autografts is still to be recommended since cognizance is thus taken of the fact that some homografts, especially in burned patients, have different survival times. Because of these variations in survival time, the likelihood of massive sloughing of homografts in any one single area would be considerably diminished. Thus the neighboring host epithelium would not have to cope with the problem of covering any large raw granulating bed at any one single time (160).

### [14] *Alteration of Donor Homograft Cells*

There are at least two major ways of approaching the problem of homografting. The simplest is to alter the homograft or *donor tissue itself* so that it becomes acceptable to the host. The other is to treat the host in such a manner that it can tolerate homograft antigens.

In general, attempts to alter the homograft itself in order to decrease its antigenicity have not been very successful. It is only within the past several years that researchers have even begun to consider that the homograft itself may react against the host by either direct or indirect means. Both Billingham (166) and Simonsen (167) have independently presented evidence at a recent international symposium which favors such an independent activity on the part of homograft cells.

Dukes and Blocker (168) reported the prolongation of survival of full thickness skin homografts by treating them with streptokinase-*streptodornase* (SK-SD) prior to transplantation. The 62 per cent longer survival time of these grafts was hypothetically due to an enzymatic débridement at the graft's cellular level to prevent early absorption by the host of nuclear material liberated by traumatization of graft cells during the usual transplantation surgery. These authors concluded that "the consistent extension of time in the enzyme-treated grafts over that of companion untreated homografts . . . indicates that investigation directed towards the graft itself . . . is a valid and feasible approach" (168).

Concerning this approach, Hardin and Werder (105) observed a greater survival rate of mouse homografts when both donor and host were *irradiated than when the host alone was so treated*. Irradiation of the donor, thus of the *homograft itself*, was thought either to attenuate the antigenicity of the graft or result in local injury of its cells sufficient to reduce its provocation of host immunity.

The effects of heat and refrigeration upon the survival of homografts, also pertinent to the general topic at hand, are discussed separately below (see [15]).

Hauschka (169) cautions that attempts to alter the graft cells are often complicated by a lack of uniformity: "conditioning . . . from without is not always a question of judicious treatment. Almost any experimental interference (by radiation, drugs, hormones, high and low temperature, antisera, storage, or ligation) is apt to favor one cell type above another and thereby modifies the graft."

### [15] *Effects of Temperature Changes on Homograft Behavior*

Many attempts have been made to reduce the "incompatibility" of skin homografts by treating them with heat (170), freezing (9?) and freeze-

drying (171) Although Sewell and his associates (171) *demonstrated slightly less fibrosis in the* host bed underlying freeze-dried homografts than *in the host bed of fresh skin homografts* there was no proof that homograft antigens had been significantly altered by the freeze-drying process In general freeze-drying destroys all viable cells in the skin graft but does not alter the mechanical properties provided by their extra cellular content *e.g.*, collagen and elastic fibers

Weisman and Cannon (170) *incubated full* thickness skin homografts and autografts of guinea pigs at 47°C *for 30 minutes* prior to transplantation this on the supposition that it would help to denature the *homografts* and thus prolong their survival The autografts survived permanently but the heat treatment apparently did not significantly alter homograft survival time Baxter and Entin (95) subjected a large number of human skin homografts to various degrees of refrigeration ranging from −45 to −108 F and concluded *from* subsequent transplantations that freezing merely shortened *the survival time of homografts, regardless of* the length of time they were stored at refrigera *tion temperatures!*

Despite the lack of success in prolonging homograft survival by heating or refrigeration the latter has provided the clinician with an excellent means of banking or storing large amounts of skin Georgiade and his associates (172) evaluating various methods of refrigera tion for the storage of skin showed that skin can be preserved for as long as seven months with *maintenance of* viable cells. These investigators demonstrated the viability of skin stored in a *glycerine-oxypolygelatin 3 per cent* potassium citrate medium up to 220 days at low tempera tures (−20 −40 −70 C) Human skin can thus be preserved for fairly long periods of time and still remain viable enough *for use as tem* porary coverage of extensively burned patients

Banking of skin assumes especially great practical importance as it bears upon large scale therapy such as might be necessary due to atomic warfare and other major holocausts It goes without saying that the temporary coverage provided the extensively burned patient by preserved viable skin homografts helps to *minimize* fluid electrolyte and protein loss *from* the burn surfaces and also reduces the *amount of* bacterial contamination In addition the patient is given a respite from the frequently painful change of dressings otherwise required in certain body sites his normal fluid balance is more rapidly restored his over all general condi tion improves and even after the homografts have sloughed away they seem *according to* some workers to have served as a stimulus to epithelization *from the* patient's own marginal epithelium Mandl and Rabinovici (173) sug gested that unknown substances originating from the graft may promote healing (neuro-hormones)

Occasionally skin is refrigerated and stored for subsequent autologous use in burned patients and in those presenting other problems of plastic and reconstructive surgery *Any excess skin removed from a donor site should nowadays be saved for the eventuality of an inadequate or poor initial take or in the event that a grafting procedure must be ended abruptly before its com pletion* Skin can also be removed from amputated limbs in traumatic cases and stored for possible *delayed coverage of the stump or other denuded* sites

### [16] *"Pretreatment" Methods to Alter Host Reaction to Homografts*

Among the methods used to depress the *host's* acquired immunity to homografts the technique of pretreating the host with small or large quantities of homologous tissue or tissue antisera has been well documented Kaliss (145) *for example* has injected small quantities of homologous tissue or tissue antisera into inbred mice prior to homotransplantation The host and donor animals were genetically unrelated Preoperative injections altered the host-graft relationships to such an extent that tumors indigenous to the donor but usually *incompatible* to and rejected by the host survived in the host and caused its death It was suggested *that the pre-injection technique does not represent* a form of desensitization but is more likely a *specific adaptive alteration of the host mouse* to the tumor tissue (115) It may be concluded *that the host mouse becomes a type A animal* insofar as its reaction to tumor graft from type A donors is concerned *In support of this* adaptive alteration hypothesis one can point to the adaptive alteration of certain micro organism (174)

*Of* special interest is the isolated report that a majority of skin homografts between unrelated mice were permanently successful when the host

animals received subcutaneous injections of homologous skin extract in specific amounts and dosages before on the day of and after the day of homografting (175) The extract was prepared from the skins of 24 mice selected at random "there was no similarity between the source of skin used as a homograft or the skin prepared as an extract for injection (175) The homologous extract was administered 3 7 or 14 days before or after the skin homografting procedures.

Similar conditions obtained when consecutive skin homografts to a given animal from the same donor were applied instead of repeated doses of homologous skin extract A single host thus received a first- second third and even a fourth-set of skin homografts from the same donor at prescribed periods of time and permanent survival of some of the transplants was recorded in a number of the cases (175)

The authors of the study did not believe that desensitization accounted for results of either the skin extract injections or the repeated grafting procedures (175) but thought rather that a state of "immunoparalysis had been induced in the host animal (176 177) In order to create such a state, the time interval between each subsequent homografting was considered important from an immunologic point of view This altered immunologic state is apparently due according to the authors to the inability of the host's antibody mechanisms to reject the homografted material Hardin and Werder (175) concluded that the immune mechanism was temporarily and in some instances permanently paralyzed by the repeated application of consecutive full thickness homografts.

Kaliss (145) and others (178) prefer to use the word "enhancement" to describe the effects of pretreating the host animal prior to homografting The term enhancement refers therefore to the prolongation of survival of tumor homografts or to the progressive growth of these grafts in mice that have received injections of mouse tissues prior to grafting In untreated mice (controls) the tumor homografts fail to establish themselves and are eventually destroyed by the immunity of the host Billingham, Brent and Medawar (179) were able to obtain increased or enhanced survival of *normal* skin homografts in mice pretreated with reconstituted suspensions of homologous lyophilized tissues taken from the same donors of the skin grafts subsequently used for transplantation

Other experimental studies with antihistamines sodium salicylate and irradiation of the recipient site by Stark, Conway and Sedar (179) have also indicated some prolongation of the average survival time of skin homografts. These are but further examples of successful attempts to alter the acquired immunity response of the host permanent survival of skin homografts however did not occur in these experiments Others have employed antihistamines (180) in skin homografting acting upon the premise that histamine is possibly liberated as a result of the antigen antibody reaction taking place between host and graft and possibly serves as a factor in the destruction of the graft Their procedures were equally unsuccessful in prolonging skin homograft survival to any significant degree

### [17] *Role of Reticuloendothelial System in Homografting*

The reticuloendothelial system (RES) has long been generally accepted to be the center for the development or formation of immunity responses in the host. Numerous workers have speculated that skin homografts would be successful if the RES could be rendered inactive Attempts to block this system by injecting various colloidal materials, *e.g* trypan blue were made on the supposition that large enough amounts of this colloid would saturate the RES so that it was no longer capable of phagocytizing colloidal particles (181) Theoretically when this ability was exhausted the RES could not perform its other normal functions, *e.g* antibody production.

Discrepancies in the results of such studies can be attributed to the erroneous assumption that all colloidal agents blocking the RES behave similarly Whereas Thorotrast and certain iron colloids can effectively block the RES for several days, trypan blue has been shown to have no effect in reducing phagocytic activity of the system (181) A technique devised to measure the radioactivity or the biologic "half life" of radioactive colloidal chromium phosphate gives a quantitative measurement of the effectiveness of the RES in removing this foreign colloid from the circulation (181) This technique has demonstrated that irradiation is effective in depressing antibody production by the RES, which contains some of the most radiosensitive tissues of

the body and is most sensitive to the effects of irradiation during the first 24 hours after the injection of antigenic material. Cortisone, like irradiation, also depresses the reactivity of the RES (see [18]).

Rabinovici (182) tried to lower the resistance of rats to homograft antigens by x-raying the animals with maximal sublethal doses prior to grafting, but he found no increase in survival time of the grafts of irradiated rats as compared to those of controls. Several years later, however, Hardin and Werder (105), using total body irradiation in young mice 6 to 8 weeks old, observed a definite prolongation of skin homograft survival, as did Conway and his coworkers (183) who irradiated only the recipient site.

Levinson and Necheles (184) obtained a markedly prolonged survival of skin homografts in rats treated with nitrogen mustard. Since the lymphocyte is thought to be one of the sites of antibody formation, nitrogen mustard probably exerts its effect on homograft survival by inhibiting the antibody action of the RES through production of a lymphocytopenia. Whereas the average survival time of rat skin homografts in these studies was 8 days for untreated controls, some grafts survived for as long as 115 days in rats pretreated with nitrogen mustard.

## [18] *Adrenocortical Hormones and Homografting*

Cortisone, hydrocortisone (cortisol) and certain other adrenocortical hormones have proved effective in prolonging the life of skin homografts in experimental animals. Billingham and his associates (101, 163) have observed that cortisone administered either systemically or locally brought about a significant prolongation of homograft survival in rabbits. Krohn (185) demonstrated in rabbits, however, that adrenocorticotropic hormone (ACTH) would sometimes prolong survival of skin homografts only in some cases and would not consistently reproduce the more efficacious results on homograft survival that are obtained when cortisone is used. These findings were corroborated by others who observed little or no beneficial results from use of ACTH (186–187).

With the exception of Whitelaw's report (188), which is open to much question, attempts to demonstrate any appreciable prolongation of survival time of skin homografts in man by the use of ACTH have been unsuccessful (103, 189, 190). Further studies by Krohn (191) and by Woodruff and Llaurado (192) have shown that corticosterone, progesterone, testosterone propionate, estradiol dipropionate and desoxycorticosterone acetate (DCA) have no direct effect on the homograft reaction (191). Systemically administered fluoro- and chloro-cortisol, prednisone and locally applied fluoro-cortisol, however, were all effective in prolonging the life of skin homografts in rabbits. It has been suggested that local application of these corticosteroids is safer than systemic administration and might prove useful in human cases (192). Rabbit skin homografts transplanted to pregnant recipients show a prolonged survival time (193), due probably to an increased production of corticosteroids by the pregnant animals.

The beneficial effects of some corticosteroids in skin homografting are attributed to their apparent ability to impair or reduce antibody function (194). Toolan showed that the administration of cortisone to hamsters (194) was effective in abolishing the hamster's normal rejection of human neoplastic tissue. Cortisone apparently conditions the host by reducing either the natural or acquired defenses against heterologous tissues. In the study at hand the heterologous tumor continued to survive after cortisone therapy was discontinued. The results of Toolan's study suggest that tumor-host relationships may be unbalanced by cortisone therapy. The combined antibody mustered by the host is overcome or overbalanced by the large amount of tumor antigen present in the host's body. In addition to its effectiveness in skin homografts, cortisone has also retarded the antigen-antibody reaction against ovarian homografts (195).

## [19] *Histochemistry of Skin Autografting and Homografting*

Little information about the metabolic changes that occur in skin autografts and skin homografts is available. Knowledge of these histochemical changes may help to clarify some of the mechanisms involved in homograft rejection (196). Studying the histochemistry of two epidermal components, ribonucleic acid and glycogen, Scothorne and Scothorne (1[illegible]) and Scothorne and Tough (91) observed that similar changes in the content of both these components occurred in the epithelium of autografts and

homografts alike during the latent period prior to homograft rejection.

Until the time of destruction of homograft epithelium changes in cytoplasmic basophilia were similar in the epithelium of both autografts and homografts Increased amounts of ribonucleic acid were observed in both types of grafts prior to breakdown of the homograft It was attributed to the increased cell growth and rate of proliferation in the grafted epithelium that occur immediately following transplantation Cytoplasmic basophilia disappeared as the homograft was rejected but was more intense in the autograft at the equivalent time period when the autograft was assuming a more normal appearance Cytoplasmic basophilia is now recognized as a sign of a high concentration of ribonucleic acid

The significance of increased amounts of glycogen in both autografts and homografts is still not clear Glycogen however is found in zones of protein synthesis of keratin between the basal epithelial layers and the granular zones ' it is reasonable to assume that its breakdown provides some of the energy for this synthetic activity (190) Because there is no major decrease in the amount of epithelial glycogen at the time of homograft breakdown Scothorne and Tough (91) concluded " it seems very unlikely that the [homograft] cells die from lack of available carbohydrate reserves.

## [20] *Acquired Tolerance and the Homograft Reaction*

One of the most challenging new concepts in the field of homograft research has been advanced by Billingham Medawar Hasek and others It is concerned with the phenomenon of acquired tolerance which can be defined as an induced state of specific non reactivity towards a substance that is normally antigenic—a non reactivity moreover that is due to a primary failure of the machinery of the immunological response (143)

The acquired tolerance and permanent acceptance of adult skin homografts by adult animal recipients can be induced artificially by exposing mice rabbits chickens or rats to living tissue antigens in the period of development before the faculty of immunological response has come into being" (143) In the case of rats this tolerance can be induced by the injection of cells from the prospective adult donor into a newborn rat soon after birth (107) This also holds true for newborn chicks (198)

In essence acquired tolerance is conferred by inoculating an animal with adult cells during its embryonic fetal or neonatal development When the animal has reached maturity homografts of any tissue subsequently transplanted from the original donor of the adult cells will then survive permanently Recent studies however demonstrate that the permanence of this tolerance is not as complete as it was earlier presumed to be Billingham (106) has shown that acquired tolerance is sometimes more complete in some mouse strains than others. Hasek (199) emphasized that acquired tolerance is not always total or complete throughout the life of an individual animal Some chickens showed tolerance for two years of their adulthood—during this time they were unable to form any agglutinins against the challenge of grafts supplied by the respective original donors of cells injected into them during their embryonic life After a third year however the capacity to form agglutinins made its appearance and another challenge of transplantation from the same donor brought about rejection of the tissue in the fashion of a typical 'homograft response Thus the established tolerance of certain foreign tissue was eventually eliminated but the progressive elimination was very slow the first signs of any rejection of the tissue were a chronic lymphocytic invasion of the graft with subsequent necrosis

Despite these demonstrations that acquired tolerance of any tissue from the same donor is not always permanent this new field of research possibly holds promise of future clinical application If in newborn infants a lasting tolerance to the tissues of merely one donor *e.g* mother or father can be induced safely it would constitute a therapeutic potential. Should the need arise in the future for any tissue or organ—skin for burns a kidney for uremia and parathyroids for hypoparathyroidism could be taken from the donor and successfully transplanted to the individual who in infancy was rendered tolerant.

## [21] *Embryonic Fetal, and Neonatal Homografts*

Tissues incompatible as homografts in their adult state are often compatible homografts when transplanted in their embryonic fetal or neonatal state The terms compatibility and

"incompatibility" could be used, therefore, merely as qualifying adjectives to describe the homografted status of ... normal adult tissue, normal embryonic tissue, benign tumor tissue, precancerous tissue and cancer" (200). An experiment which illustrates the compatibility of normal fetal tissue involved the successful homotransplantation of fetal skin from black rats to adult white rats. When this skin was buried subcutaneously (see [22]) and subsequently brought to the surface, in some cases it appeared to survive indefinitely (201).

Reports of "acquired tolerance" and of the survival of embryonic skin, for example, certainly indicate that the term "incompatibility" is no longer universally applicable to homografts. The "incompatibility" of adult normal tissues when homotransplanted to other adults, can be abolished by inducing "acquired tolerance" in the host. These two new channels of research, i.e. acquired tolerance and the use of embryonic, fetal, and neonatal donor tissue, strongly demonstrate the marked differences that exist between the immunity mechanisms of embryonic and adult hosts and donors. The use of embryonic, fetal or neonatal tissues to repair burned surfaces (202–203) and endocrine deficiencies (204–206) indicates that successful homotransplantation, as measured by clinical feasibility, can be attributed in part to the increasing awareness of the differences in the "compatibility" and "incompatibility" of embryonic and adult tissues.

Recent clinical and experimental evidence suggests that some embryonic, fetal, and even neonatal tissues do not have at the time of their removal from the donor an absolute tissue specificity (198). In addition, certain animal species show a period of immunologic "neutrality" during which they apparently accept these tissues rather than reject them violently as in the case of most adult hosts with most adult tissue homografts. As an example of this, Cannon (198) has shown that approximately five to ten per cent of skin homografts taken from one-day-old chickens and transplanted to one-day-old chickens are permanently successful.

These data have interesting implications. It can probably be concluded that the tissue specificity of skin of a one-day-old chicken is not yet absolute and therefore is alterable as is the immune mechanism of a one-day-old chicken or embryonic chicken host. It is pertinent to consider what evidence is available of altered behavior of embryonic, fetal or neonatal tissues used as donor material.

In a pilot case by Peer and Rogers reciprocal skin homografts were exchanged between a mother and her three-month-old son. Subsequent studies by Peer and his associates (115) demonstrated the surprising finding that skin taken from infants two to three months old and transplanted behind their mothers' ears survived for periods (limited by the date of their report) of 56, 140, 209 and 321 days; none has been rejected at the time of the present writing. These results are particularly impressive in view of data showing that the average survival time of skin homografts exchanged between middle-aged, non-related human males ranges only from 7 to 9 days (114). The report of Peer and his associates (115) supports the findings of Cannon (198) in that it suggests that neonatal or even young infant (donor) tissue apparently lacks the absolute specific antigenicity characteristic of adult tissues. Of course, it cannot be denied that the genetic similarity of donor and host (infant and mother) also partially accounts for the increase in survival time (114), but when one considers that survival times range between 19 and 29 days for homografts between such genetically similar individuals as adult dizygotic twins (114), the much greater survival time in Peer's studies is noteworthy.

Also relevant to an evaluation of the behavior of skin in an early stage of development are the findings of Helsingen and Helsingen (207), who took fetal skin from a stillborn baby delivered three weeks prior to term and transplanted it to an unrelated severely burned child. Its survival time, although definitely prolonged as one would expect in a burned individual (114), was approximately seven weeks. At the end of this period, the fetal skin grafts had dissolved, and no remaining grafts could be seen.

In the light of recent reports by Toolan (202) and Gaillard (203), it becomes obvious that the state of development of an embryo or fetus largely determines the reception of embryonic or fetal skin by the adult host. Whereas fetal skin taken from a stillborn (207) 3 weeks prior to term survived for only 7 weeks even in the presence of increased corticosteroid of a burned patient, fetal skin taken by Gaillard (203) from a 3-month-old human fetus and placed upon a burned patient has had a survival to date of more than 6 months. In addition, fetal skin

taken from human fetuses approximately in the fourth month of gestation and transplanted by Toolan and her associates (at the Surgical Research Unit at Brooke Army Medical Center Fort Sam Houston Texas) to severely burned individuals has had an apparent survival time, to date of more than one year

The long term survival of skin grafts obtained from fetuses 3 to 4 months old as contrasted with the briefer survival of skin grafts from fetuses stillborn just prior to term suggests that tissue antigen specificity is the less absolute as implied by Cannon (198) the earlier the stage of development of the (donor) embryo or fetus. Toolan's recent experiments (202) demonstrate that the ideal age for procurement of human fetal skin is probably in the first trimester of pregnancy Toolan has corroborated these findings in similar experiments with rabbits (208)

Consonant with the conclusions of Cannon (198) and Toolan (202) Rogers, Converse and Silvetti (209) are studying bovine embryo skin as a possible substitute for skin homografts in some clinical conditions, especially mass emergencies. The fresh bovine embryo skin stored at refrigerating temperature (4°C) in Ringer's solution has had a total survival time varying from 12 to 17 days in human volunteers. This is almost comparable to the survival time of freeze-dried cadaver skin homografts obtained from the pioneering U S Navy Tissue Bank set up by Commander George Hyatt at Bethesda Maryland. This physical presence or survival time of 12 to 17 days indicates that these grafts when used in the fresh refrigerated condition are practical as temporary biologic dressings for certain types of defects created during long term reconstructive procedures in plastic surgery—*e.g.*, temporary scalp defects created when forehead full thickness skin flaps are swung down from the frontal-parietal or temporal regions to reconstruct missing portions of the nose. The embryonic heterografts serve these cases as a temporary covering of the scalp defect for the interval required before the flap is returned from its position on the nose to its former bed on the scalp

Freeze-dried bovine embryo skin grafts show survival times that to date in the studies under way are definitely inferior to the survival of freeze-dried cadaver skin homografts The total survival time or physical presence of these bovine graft on defects in human volunteers ranges only from 7 to 10 days. It is possible that the freeze-drying process is too damaging to the sensitive thin embryo skin, which has little or no true stratum corneum as a form of mechanical protection from the dressings applied to it when it is used as a graft. However preliminary studies suggest encouraging results with this freeze-dried skin when used in treating small decubitus and varicose ulcers. In these conditions it seems to have stimulated epithelization from marginal and from epithelial islands in the ulcers themselves thus paralleling the effect attributed to the action of fetal membranes when used in similar conditions.

### [22] *Fetal Membranes as Homografts*

Experimental work undertaken by Douglas and associates (210) suggests that homologous and heterologous fetal membrane grafts are tolerated just as well as skin homografts when used as biologic coverings for open wounds. In many cases they seemed to be tolerated for two to three times the average survival time of skin homografts. The authors in question demonstrated that fetal membrane transplants in mice were apparently viable and capable of undergoing cellular division and epithelization They concluded from their preliminary studies that human chorionic grafts and other placental grafts might conceivably be more practical as a temporary covering for burns and other types of wound defects than skin homografts.

More recently Troensegaard-Hansen (211) has reported encouraging results with the use of human amnion in treating chronic unresponsive varicose ulcers. The amnion is usually boiled prior to its implantation, and although it disappears in several weeks after application to the ulcer it seems to encourage a rapid epithelization from the periphery of the ulcer It was suggested that the amnion in these cases provides a biologic surface along which marginal epithelial cells can migrate and also that it possibly liberates chemical substances which might specifically stimulate epithelial growth Other cases in which stimulation of or regeneration from marginal islands of epithelium has resulted from the application of amniotic membrane grafts as dressings for severe burns are described by Sterling (212) His report is a follow-up of studies also made by Douglas in 1952 (213) Douglas observed that amniotic membrane grafts placed on similar wounds seemed to be converted into a

mesodermal hyaline fibrinoid type of tissue membrane which served as a satisfactory temporary covering or tissue envelope.

Other research in recent years has recorded the successful transplantation of fetal and neonatal endocrine tissue in adults suffering from various endocrine disorders or deficiencies. The results of these studies are very encouraging and fortify the promising nature of results described in the foregoing sections on homotransplantation of embryonic, fetal, and neonatal skin and membranes. Gaillard, for example (203), reported the successful transplantation of parathyroid tissues taken from newborns or from stillborn human fetuses. Following two to three weeks of tissue culture in the serum of the recipient host, the glands were transplanted to the perivascular spaces of the brachial artery or vein in patients with hypoparathyroidism. All patients in Gaillard's report were in a hypoparathyroid state with tetany as a result of too extensive a surgical thyroidectomy. Following transplantation their hypoparathyroid tetany was corrected, calcium levels rose, and phosphorus levels dropped. Best results were obtained in young patients between the ages of 16 and 25.

Woodruff (214) of Edinburgh has transplanted fetal adrenal glands to the rectus abdominal muscle of two patients suffering from Addison's disease. Prior to transplantation both patients required DOCA implantation and corticosteroid therapy. *Subsequent to implantation no further* DOCA or steroids were required. Jones (206) reported the apparently successful homotransplantation of fetal ovaries chopped into pieces roughly 2 to 3 mm. in diameter. The grafts were implanted into both rectus muscles of a 34-year-old woman suffering severe menopausal symptoms resulting from a surgical gonadectomy (bilateral oophorectomy and hysterectomy). Prior to homografting none of the symptoms responded to conventional hormonal therapy. Four weeks after ovarian homografting, her symptoms had been relieved, and this relief was maintained for at least 5 months when Jones made his report.

### [23] *Variations of Autograft and Homograft Behavior in Other Sites*

The Woodruffs (215), Medawar (216), Greene (217) and others have shown that certain transplantation sites in the body might be called "privileged sites," since they are apparently free of the usual homograft rejection phenomena. The anterior chamber of the eye and the brain are examples of such sites and skin homografts transplanted to them will survive permanently or indefinitely, lasting throughout the lifetime of the host. The anterior chamber of the eye is apparently the most favorable, with the white matter of the brain an almost equally "privileged" site. Skin homografts and other tissue homografts survive in these sites, however, only if they remain unvascularized (216). Adult homografts transplanted to many other sites, *e.g.*, muscles, blood vessels, joints, subcapsular space of kidney, et cetera, rarely survive permanently and are almost always rapidly destroyed. Because the anterior chamber and the brain have little if any lymphatic drainage, Medawar concludes:

> It is suggested that the presence of a lymphatic drainage system is necessary for immunity to be called into being and that penetration by blood vessel must occur (see [24]) "before it can come into effect. ... A skin homograft transplanted to the anterior chamber of the eye of a specifically immunized rabbit is destroyed if, and only if, it is penetrated by blood vessel. ... Skin homografts transplanted to the brain submit to but cannot elicit an immune state. ... Skin homografts transplanted to the brains of specifically immunized rabbits respond by total breakdown to an immune state already in being. On the testimony of other workers, they enjoy prolonged or *indefinite survival when grafted to the brain of* non-immunized animal ... (216)

The permanent survival of skin autograft when transplanted to another skin defect is generally taken for granted. When skin is grafted to other sites in the same body, however, some of its cellular components undergo a radical change and may even degenerate.

#### BURIED AUTOGRAFT

*Subcutaneous burial of autografts* has particularly interested clinicians because of their possible application to the repair of large facial and other contour defects. These defects are common in some major operative procedures in *which a recurrent ventral hernia is the chief* complication and symptom. Some surgeons believe that skin autografts are particularly indicated in those patients in whom it is desirable to use autologous tissue for repair rather than *some foreign inert material, e.g.*, tantalum mesh.

At this point it should be stressed that a

cutis or "dermal" graft is differentiated from a buried full thickness graft in that the former is a piece of skin or graft from which the epithelium or epidermis has been removed leaving intact the dermis only. It is interesting from the viewpoint of history to note an astute clinical observation by Reverdin: he was one of the first to suggest that epithelial cysts developed when small pieces of epidermis were torn off during trauma and implanted deeply into the dermis (218). Several years later Garré suggested that burial of epidermis by itself produced a cyst with smooth walls, whereas a buried full thickness autograft (including epithelium and dermis) produced a cyst that also contained papillae.

The development of these cysts is to some surgeons a deterrent to the use of buried skin autografts in the repair of hernias, etc. However, much clinical and experimental work in the last half century has shown that in most cases the cysts are eventually absorbed and after a considerable period of time, they can no longer be found upon biopsy. The latter observations were made especially clear in the works of Peer and Paddock (219-220).

In 1939 Peer studied the behavior of full thickness skin autografts taken from the thoracic region and buried in the thoracic area together with their subcutaneous fat. Microscopic sections were made of biopsied material removed at intervals ranging from two weeks postoperatively to 28 months (fig. 60). Epidermis survived in

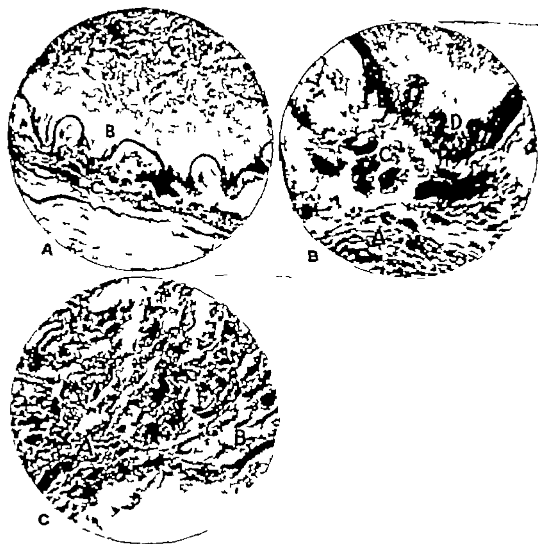



Fig. 60. Behavior of buried full thickness skin autograft. A. At one month epidermis is thin and in the process of absorption by giant cells. Sebaceous glands are gone. B. High power magnification of buried skin at one month. Note giant cells phagocytizing the epidermis. C. Only the dermis is surviving in full thickness skin graft buried 28 months. (From L. A. Peer and J. C. Walker, Jr.: The behavior of autogenous human tissue grafts: comparative study, parts 1 and 2. Plast. & Reconstruct. Surg. 7: 6, 73, 1951.)

grafts two weeks old and one month old but in sections of all subsequent biopsied tissue epidermis was entirely absent Similarly sebaceous glands were still detectable in tissue biopsied two weeks after operation but even at this early date the glands showed distinct degenerative changes. Upon later biopsy no sebaceous glands could be observed at all Hair follicles seemed to survive for a somewhat longer period and were seen in material taken as late as 5½ months after operation. Later biopsies however failed to demonstrate any hair follicles Interestingly sweat glands were still detectable in all biopsies from the time of operation up to the twenty eighth postoperative month. Autografted dermal fibroblast cells however and autografted collagenous and elastic fibers, since they are "buried in their natural state apparently survived as such in these buried dermal and full thickness skin autografts (219–220)

Laboratory reports of cyst formation from buried skin grafts in dogs led Peer to suggest that full thickness autografts buried in the subcutaneous tissue of man behave differently from similar full thickness autografts buried in dogs. In studies by Peer and Paddock (219) in which small pieces of abdominal cutis" grafts were buried beneath the thoracic skin of human volunteers, microscopically small cysts developed from the epidermal appendages that lay deep in the dermis. They remained for at least two months following autotransplantation. In later biopsies, however the small cystic cavities seen twelve months postoperatively no longer had any epithelial lining, but their cystic cavities were filled with an epithelial debris. Peer concluded (220) It seems apparent that small bits of epidermis produce less reaction in the surrounding host tissue and survive long enough to form small cystic cavities Eventually however the epidermis is completely absorbed."

The studies of Peer and Paddock (219–220) are clinically valuable since from a practical standpoint their findings differ markedly from those of comparable work with animals. Zimches (221) for example who followed buried skin graft in dogs for as long as two years after surgery was still able to find cysts with epidermal lining and they were actually increasing in size These cysts of course would be a disadvantage in man since their continuous growth would interfere with reparative processes and they would serve as weak point in the dermal support provided by a buried *graft to a fascia defect* Peer concluded from his observations (220)

> The most valuable information obtained from the experiments was the fact that autogenous skin *buried in human beings acts differently from autogenous skin buried in animals* (guinea pig dog and rabbit)

In later experiments Swenson verified the findings of Peer and Paddock by burying cutis grafts in dogs for periods varying from 7 to 152 days (222) He observed the primary histologic change to be a degeneration of the epithelial elements. Since the pioneering work of Peer the use of "dermal" grafts has increased in popularity Buried dermal grafts are now employed for the repair of large ventral hernias (222) as an alternative to tubed flaps or direct skin flaps (223) for the repair of benign strictures of the bronchus and trachea (224) as filling material in extensive tissular and contour defects (225) etc Their use however is not always free of complications. The risk of cyst formation despite the experimental findings of others (219–220 222) must never be forgotten in performing this type of surgery Gibbon and Lothian (226) described two cases in which gross cyst formation occurred with survival of sweat and sebaceous glands and hair follicles following the use of dermal grafts for inguinal hernia repair Rosenblatt and his associates (227) however emphasized that the risk of cyst formation is considerably less when cutis grafts are used in preference to full thickness grafts. Cyst formation occurs in approximately 5 per cent of dermal autografts (228)

Clinically buried full thickness autografts *have been employed for the repair of incisional* hernias (225 229) and eventrations (229) joint ligaments (230) as a substitute for fascial sutures (231) etc Peer and Walker (228) observed

> Since the hair follicles in buried skin grafts degenerate earlier than the Malpighian cell in the epidermis it is possible to destroy the hair follicles by burying the skin for a period of two weeks and then retransplanting the skin *graft* on the surface The epidermis will readily regenerate but the hairs will not reappear

Swenson and Lee (232) recommend that freeze-dried dermal and full thickness skin homografts might be applied to the field of congenital body wall defect such an absence

of the hemidiaphragm and omphalocele and many others.

Split-thickness buried autografts have been used experimentally to control liver hemorrhage (233), split-thickness full thickness and dermal autografts have been transplanted successfully to the thoracic cavity (234) and to the peritoneal cavity (235–236) where each respectively grows well when fixed securely to the lung, heart, pericardium, diaphragm trachea esophagus parietal pleura, thoracic aorta parietal and visceral peritoneum, the outer surfaces of intestine and urinary bladder etc. The implications of these experimental studies on future advances in thoracic and abdominal surgery are self-evident!

### [21] *Cell Survival Versus Cell Replacement in Autografting*

Whether autografted cells survive whether they are completely replaced by host tissues or whether there is a combination of those processes are still unsettled questions. Some workers stress the numerous differing interpretations of this problem. Hyatt, discussing Peer's cellular survival theory (238) (237) stated

> In view of the difficulty of determining the origin of cells and in determining viability of a cell one must ask for further information as to the methods of determining the cellular origins How was it determined that the 'viable cells were from the original graft and not from the host? When rib tibia or iliac grafts are transplanted into *soft* tissue it is impossible to determine whether the cells survive or are replaced by the matrix in our opinion

Peer however (238) emphasized

> 'The theory of gradual replacement of free autogenous grafts by infiltrating host cells in such a manner that bone is replaced as bone cartilage as cartilage fascia as fascia and tendon as tendon has dominated the thinking of past investigators and still influences the reasoning of present-day experimenters and clinicians Actually it is possible that *the replacement theory is without foundation with respect to most human autogenous tissue grafts and is known to apply only to some bone grafts and to nerve grafts to a limited extent*
>
> After careful study of the behavior of ten commonly used free autogenous tissue grafts in humans the author concluded that the great majority of these grafts probably survive and are not replaced by host tissue cell This general tendency for the cells in free grafts to remain viable after transplantation is described as the *cell survival theory* (238) This theory is postulated as follows *In humans the cells in free autogenous grafts tend to survive and retain their normal tissue structure when transplanted as complete cell entities in favorable transplantation sites When the cells in free grafts fail to survive the graft is replaced by connective tissue but this replacement is not a duplicate of the original graft*

Peer then goes on to describe his observations with autografts of skin

> 'The ectodermal portions of split or full thickness skin grafts are not replaced by infiltrating host epidermal cells or host fibroblasts This is easily demonstrated by the survival of hairs and glands in the dermis and the fact that pigmented nevi and other growths remain after transplantation The fact that angiomas remain in skin grafts following transplantation indicates that the vascular system survives Positive evidence from microscopic examination of skin grafts at intervals from one to 14 days indicates rather strongly that the fibroblast cells in the dermis survive and are not replaced by host fibroblasts

Although the foregoing quotations deal largely with cellular components other than vasculature, they are indicative of the 'survival (238) versus 'replacement (237) argument Both camps have an apparently good stock of personal clinical and experimental data to support their cases. As is usually the rule however the answer probably lies somewhere between these two poles of opinion. This seems to be indicated when one considers the available information on the behavior of autograft vasculature.

### *Survival or Replacement of Skin Autograft Blood Vessels?*

In 1925 Davis and Traut (239) summarized the findings of previous workers in this field and added their own new data on the origin and development of blood supply in skin grafts Davis and Traut, and Converse and coworkers (75) pointed out that the vascularization of skin grafts both autologous and homologous is generally accepted as a combination of three processes 1) direct connections between graft vessels and host vessels 2) ingrowth of host vessels into the graft's endothelial channels and 3) penetration of host vessels into the graft's dermal connective tissue, thus forming new endothelial channels. It has been assumed that

prior to this revascularization the graft is nourished by a poorly defined process known as "plasmatic circulation" (240).

Many surgeons observing the behavior of skin grafts in their patients within the first few hours after transplantation have been impressed by characteristic color changes in the graft. When the graft is removed from the donor site it usually becomes white and blanched. Within a few hours after transplantation to the host bed it takes on a pinkish hue which then progresses to a bright pink color during the first few days following transplantation. Douglas (241) noted that grafts became slightly pink as early as eight hours after transplantation. Hynes (242) studying human skin homografts observed that a graft contracts upon itself after its removal from a donor site and expels from its vessels most of the formed hemic elements which they contained. Within twenty-four hours after transplantation the graft vessels are dilated although they contain only a few hemic elements. Within forty-eight hours, however, the graft vessels contain large numbers of red blood cells.

Preliminary observations of this phenomenon (240) by Converse and his associates suggest that skin grafts are capable of absorbing fluid from the host bed due to the sponge-like structure of their dermis, which is canalized by innumerable endothelial spaces and lumina. Fluid from the host bed may be absorbed in a manner which might be compared to that of blotting paper. This rapid absorption of plasma-like fluid may account for the color change in the graft. An early filling of the endothelial spaces of the graft with plasma-like fluid is accompanied by the infiltration of only a few erythrocytes. After twenty-four hours, however, the graft contains erythrocytes in larger numbers, the result of anastomosis of a few graft vessels with a few host vessels coupled with the early ingrowth of host endothelium. Penetration of the graft by red blood cells is probably partly responsible for the development of a pink color in the graft. In Finerty's review (243) of parabiosis (the union of two experimental animals or human beings by a cross pedicle skin flap with subsequent direct capillary anastomoses between the two individuals) he concluded that parabiotic studies show the beginning of capillary union occurs between the second and third postoperative days and proceeds rapidly thereafter. In some experiments (244-245) vascular inosculation was conclusively demonstrated by a transfer of significant quantities of red blood cells between the parabionts as early as two hours after surgical union was established.

Plasmatic circulation can probably be described, therefore, as a period during which the graft vessels fill with fluid and cells from the host bed. The term circulation is actually a misnomer because the fluid absorbed by the graft from the host bed is trapped within the graft. Ingrowth of endothelial buds from the host and direct connection of graft vessels and host vessels are concomitant processes that are probably initiated as soon as the initial lag period in wound healing is overcome at the host-graft junction. Endothelial ingrowth from the host progresses until definitive vasculature is established. The stagnant fluid absorbed by the graft during the early phase of "plasmatic circulation" is probably drained off by the reestablishment of a definitive blood and lymphatic circulation (240).

Converse and his associates (75) believe that ingrowth of host vessels into autograft and homografts is essential for the establishment of the definitive vasculature of the graft. They base their opinions on the observations that during the first days after transplantation the graft vessels contain formed hemic elements. The fate of many of these vessels, however, appears to be the same: their contained blood cells are hemolyzed, the nuclei of their lining endothelial cells become pyknotic, then lysed, and finally the structures can no longer be recognized as blood vessels. Rogers, Converse and Taylor (70) made similar observations in skin autografts in 1951.

*In vivo* stereomicroscopic observations (73) of human skin autografts and homografts revealed dilated vessels containing formed hemic elements during the first few days after transplantation; these dilated vessels exhibited no hemic flow. During subsequent days in the autograft the dilated vessels appeared to be replaced by finer calibered vessels (fig. 61) which established active blood flow between the graft and the host bed. Early in the postoperative period the vessels of the graft itself appeared to serve a temporary function, only filling with fluid and cells and then gradually disappeared. Most of the definitive vasculature of the graft appeared to be constituted

by vessels ingrown from the host which thereby established hemic flow between graft and host.

Although the suggestions of Peer (238) concerning his *cell survival theory* must not be dismissed too quickly in view of the above findings one must take exception to at least one of his statements namely "The fact that angiomas remain in skin grafts following transplantation indicates that the vascular system survives" (238) Numerous studies suggest that, actually, continued survival of angiomas in skin autografts could just as easily be explained by the observed phenomena previously described—that is the vascular channels of the graft's angioma or the angioma "pattern" of vessels could be reestablished or reduplicated in essentially their original form by any one of the three methods of vascularizing of skin grafts described by Davis and Traut (239) 1) direct connections could first be made between host vessels and the angioma's vessels 2) ingrowth of host vessels into the angioma's endothelial channels could take place and 3) host vessels could penetrate into the graft and into the angioma's intervening dermal connective tissue thus forming new endothelial channels. Since these three processes have been well authenticated in recent studies of autografts and homografts, Peer's conclusions that "the vascular system survives" again points up the continuing controversy that goes on between proponents of survival and proponents of cell replacement in autogenous tissue

The observation that non-viable freeze-dried skin homografts are penetrated by new blood vessels from the host although at a slower rate than occurs in viable grafts (75) leads to the further conclusion that, *as the vessels of non-viable tissue are incapable of serving any function other than that of conductors for ingrowing host vessels, revascularization does not appear to be entirely dependent upon survival of graft vessels* (75)

In addition the new vasculature of an autograft is of a coarser pattern initially than that of normal skin (240) The degeneration of graft vessels and the incomplete early rehabilitation of the endothelial spaces by the ingrowing host vessels are an explantation of the relative coarseness. In the subsequent postoperative days the graft vasculature progressively resumes a finer pattern approximating that of normal skin (75) Medawar observed the same kind of phenomena in his bi le studies of skin autografts in rabbits (8)

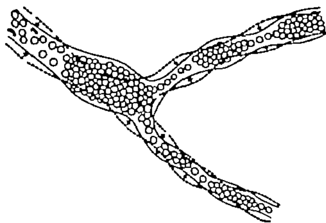


FIG 61 Diagram made from stereomicroscopic observations illustrating changes of caliber of minute subpapillary vessels of normal skin as a result of hemic flow The passage of the formed hemic elements through the vessels causes changes in caliber of the vessels the diameter of the vessels is directly proportional and the rate of the flow is inversely proportional to the cell mass passing through them. (From J M Converse and F T Rapaport The vascularization of skin autografts and homografts an experimental study in man Ann Surg 143: 306 1956 )

During the time interval between implantation of the autograft and revascularization many cells populating the graft dermis lose their affinity for stains so that portions of the reticular and papillary layers of the dermis appear cell free (70 75) These apparent degenerative changes in grafts have also been described by Davis and Traut (239) With the reappearance of vessels cells again populate the collagenous and elastic reticulum of the graft. Neotetrazolium enzymatic activity in grafts shows the return of cellular activity occurring first around the newly ingrown vessels (75) It is not clear whether these cells are proliferating from surviving graft cells or have emigrated into the graft with the ingrowing host vessels Here again with many fine histologic points still unsettled, the controversy of cellular survival versus cellular replacement is still a legitimate debate Methods to establish the continued survival or viability of autografted or homografted vasculature and other cells, such as the use of supravital histologic staining are still crude and inconclusive The most practical advance in "viability studies" made in recent years however *was contributed* by Peer (64) who ingeniously made use of the female sex chromatin in epithelial cells to establish the continued survival of female

prior to the revascularization the graft is nourished by a poorly defined process known as plasmatic circulation (240).

Many surgeons observing the behavior of skin grafts in their patients within the first few hours after transplantation have been impressed by characteristic color changes in the graft. When the graft is removed from the donor site it usually becomes white and blanched. Within a few hours after transplantation to the host bed it takes on a pinkish hue which then progresses to a bright pink color during the first few days following transplantation. Douglas (241) noted that grafts became slightly pink as early as eight hours after transplantation. Hynes (242) studying human skin homografts observed that a graft contracts upon itself after its removal from a donor site and expels from its vessels most of the formed hemic elements which they contained. Within twenty-four hours after transplantation the graft vessels are dilated although they contain only a few hemic elements. Within forty-eight hours, however, the graft vessels contain large numbers *of red blood cells*.

Preliminary observations of this phenomenon (240) by Converse and his associates suggest that skin grafts are capable of absorbing fluid from the host bed due to the sponge-like structure of their dermis which is composed of innumerable endothelial spaces and lumina. Fluid from the host bed may be absorbed in [illegible] which [illegible] paper. Th[illegible] fluid may [illegible] the [illegible] graft. An [illegible] the graft [illegible] fluid [illegible] by the [illegible] After [illegible] however, the graft contains [illegible] in larger numbers, the result of [illegible] a few graft vessels with a few [illegible] coupled with the early ingrowth [illegible] Penetration of the graft by red blood cells is probably partly responsible for the development of a *pink color in the graft*. In Finerty's review (243) of parabiosis (the union of two experimental animals or human *beings by a cross pedicle skin flap with subsequent direct capillary anastomosis between the two individuals*) he concluded that parabiotic studies show the beginning of capillary union occurs between the second and third postoperative days and proceeds rapidly thereafter. In some experiments (244–245) vascular inosculation was conclusively demonstrated by a transfer of significant quantities of red blood cells between the parabionts as early as two hours after surgical union was established.

"Plasmatic circulation" can probably be described therefore as a period during which the graft vessels fill with fluid and cells from the host bed. The term "circulation" is actually a misnomer because the fluid absorbed by the graft from the host bed is trapped within the graft. Ingrowth of endothelial buds from the host and direct connection of graft vessels and host vessels are concomitant processes that are probably initiated as soon as the initial lag period in wound healing is overcome at the host-graft junction. Endothelial ingrowth from the host progresses until definitive vasculature is established. The stagnant fluid absorbed by the graft during the early phase of "plasmatic circulation" is probably drained off by the reestablishment of a definitive blood and lymphatic circulation (240).

Converse and his associates (75) believe that ingrowth of host vessels into autografts and homografts is essential for the establishment of the definitive vasculature of the graft. They base their opinions on the observations that during the first days after transplantation the graft vessels contain formed hemic elements. The fate of many of these vessels, however appears to be the same: their contained blood cells are hemolyzed, the nuclei of their lining endothelial cells become pyknotic, then lysed and finally the structures can no longer be recognized as blood vessels. Rogers, Converse and Taylor (70) made similar observations in skin autografts in 1951.

*In vivo* stereomicroscopic observations (73) of human skin autografts and homografts revealed dilated vessels containing formed hemic elements during the first few days after transplantation; these dilated vessels exhibited no hemic flow. During subsequent days in the autograft the dilated vessels appeared to be replaced by finer calibered vessels (fig. 61) which established active blood flow between the graft and the host bed. Early in the postoperative period the vessels of the graft itself appeared to serve a temporary function only, filling with fluid and cells and then gradually disappeared. Most of the "definitive" vasculature of the graft appeared to be constituted

by vessels ingrown from the host which thereby established hemic flow between graft and host.

Although the suggestions of Peer (238) concerning his *cell survival theory* must not be dismissed too quickly in view of the above findings, one must take exception to at least one of his statements, namely "The fact that angiomas remain in skin grafts following transplantation indicates that the vascular system survives" (238). Numerous studies suggest that, actually, continued survival of angiomas in skin autografts could just as easily be explained by the observed phenomena previously described—that is, the vascular channels of the graft's angioma, or the "angioma pattern" of vessels could be reestablished or reduplicated in essentially their original form by any one of the three methods of vascularizing of skin grafts described by Davis and Traut (230): 1) direct connections could first be made between host vessels and the angioma's vessels, 2) ingrowth of host vessels into the angioma's endothelial channels could take place, and 3) host vessels could penetrate into the graft and into the angioma's intervening dermal connective tissue, thus forming new endothelial channels. Since these three processes have been well authenticated in recent studies of autografts and homografts, Peer's conclusions that "... the vascular system survives" again points up the continuing controversy that goes on between proponents of survival and proponents of cell replacement in autogenous tissue.

The observation that non-viable freeze-dried skin homografts are penetrated by new blood vessels from the host, although at a slower rate than occurs in viable grafts (75), leads to the further conclusion that *as the vessels of non-viable tissue are incapable of serving any function other than that of conductors for ingrowing host vessels, revascularization does not appear to be entirely dependent upon survival of graft vessels* (75).

In addition, the new vasculature of an autograft is of a coarser pattern initially than that of normal skin (246). The degeneration of graft vessels and the incomplete early reinhabitation of the endothelial spaces by the ingrowing host vessels are an explanation of the relative coarseness. In the subsequent postoperative days the graft vasculature progressively resumes a finer pattern approximating that of normal skin (75). Medawar observed the same kind of phenomena in his basic studies of skin autografts in rabbits (8).

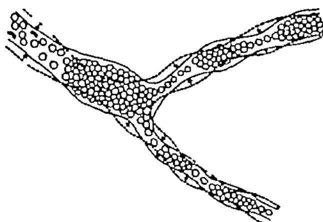


Fig. 61. Diagram made from stereomicroscopic observations illustrating changes of caliber of minute subpapillary vessels of normal skin as a result of hemic flow. The passage of the formed hemic elements through the vessels causes changes in caliber of the vessels; the diameter of the vessels is directly proportional, and the rate of the flow is inversely proportional to the cell mass passing through them. (From J. M. Converse and F. T. Rapaport: The vascularization of skin autografts and homografts: an experimental study in man. Ann. Surg. 143: 306, 1956.)

During the time interval between implantation of the autograft and revascularization, many cells populating the graft dermis lose their affinity for stains so that portions of the reticular and papillary layers of the dermis appear cell free (70, 75). These apparent degenerative changes in grafts have also been described by Davis and Traut (230). With the reappearance of vessels, cells again populate the collagenous and elastic reticulum of the graft. Neotetrazolium enzymatic activity in grafts shows the return of cellular activity occurring first around the newly ingrown vessels (75). It is not clear whether these cells are proliferating from surviving graft cells or have emigrated into the graft with the ingrowing host vessels. Here again, with many fine histologic points still unsettled, the controversy of cellular survival versus cellular replacement is still a legitimate debate. Methods to establish the continued survival or viability of autografted or homografted vasculature and other cells, such as the use of supravital histologic staining, are still crude and inconclusive. The most practical advance in "viability studies" made in recent years, however, was contributed by Peer (61) who ingeniously made use of the female sex chromatin in epithelial cells to establish the continued survival of female

homograft on male hosts Obviously the converse the absence of this chromatin in male epithelial cells can also be used a an indicator of viability or continued survival of a male skin homograft transplanted to a female host

Attempts to use tattooing or pigmented cells to indicate continued survival or viability of graft are also relatively inconclusive Conway and Sedar (217) for example recently showed that a loss of pigmentation occurred in the hair of autograft successfully transplanted from the dorsal to ventral region of mice They concluded that graft melanoblasts were destroyed by the transplantation procedure

### *The Rate of Revascularization of Autografts and Its Effects*

It has been recognized that split thickness autografts are revascularized more rapidly than full thickness graft in man (75) The histologic examination of both types of graft reveals that degenerative changes in the graft dermis vary according to the rate of revascularization. When revascularization is fairly rapid, degenerative changes in the dermis are minimal. And such changes are less prominent in split thickness grafts because the invading blood vessels have a shorter distance to travel to penetrate the entire graft. Thus, cellular survival appears to be partially dependent upon rapid revascularization (75)

Revascularization is more rapid when the graft is derived from a richly vascularized donor site and is applied to a well vascularized host bed This would also explain the rapid filling or revascularization of the angioma in the skin autograft described by Peer (7) The success of "composite" chondrocutaneous grafts from the ear in the reconstruction of nasal defects may be explained on this basis (fig. 62) Human full thickness auricular skin grafts applied to the chorioallantoic membrane of a chick embryo appear to be revascularized more rapidly than full thickness grafts derived from other donor

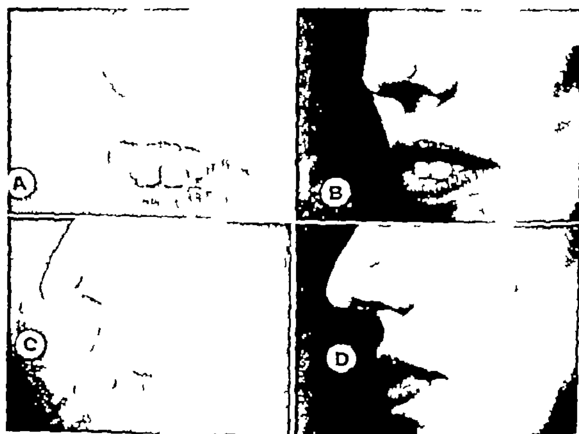



FIG 62 B Previously excised at another clinic this recurrent nasal alar lesion extended down to alar cartilage Diagnosis basal cell carcinoma A Full thickness wedge excision of entire thickness of nose including skin underlying cartilage and nasal mucosa left a defect filled with a "composite" full thickness graft taken from left ear C Profile view of recurrent basal cell carcinoma of left nasal alar region requiring through and through wide wedge excision D Profile view of nasal alar defect filled with a composite full thickness skin graft taken from left ear

sites (75) These observations may explain the rapid revascularization of experimental grafts on the rabbit's ear and the apparent survival of graft vessels

In conclusion, an acceptable settlement of the controversy of cell survival versus cell replacement in autografts is dependent upon a multiplicity of factors e.g slow revascularization, rapid revascularization etc The foregoing discussion shows that Peer's cellular survival theory" is thus partially substantiated but so also is the theory" of cellular replacement. In a considera tion of any one single autograft of skin therefore, it may not be amiss to suggest that *most of the autograft vessels are replaced by host vessels that graft collagen and elastic fibers probably survive intact, that some graft dermal cells are replaced and some survive that graft epidermal appendages e g hair follicles and sebaceous glands survive in many instances and that graft epithelium survives if it soon receives an osmotic nourishment by a rapid revascularization of its underlying graft dermis but it may in part be replaced by marginal host epithelial ingrowth if its nourishment is too slow or poor*

## CLINICAL USES FOR SKIN AUTOGRAFTS AND HOMOGRAFTS

The two chief uses for skin homografts at the present, if adult skin is the donor material, are 1) the "temporary coverage of severe burns, and 2) the 'temporary coverage of skin defects created during the reconstructive repair of an area lacking skin, such as a forehead defect created by temporary rotation of a scalp flap for repair of a nasal deformity In this instance freeze-dried banked human cadaver skin is a satisfactory covering for approximately 16 to 18 postoperative days. In some patients with congenital agammaglobulinemia (248) experi mental skin homografts apparently surviv permanently because of a failure in the patient's immune responses to antigenic stimuli but the practical clinical importance of this finding is minimal

Conway and Stark (249) ingeniously used the reaction of skin homografts purposely to provoke multiple thromboses of a bleeding wound in a patient suffering from hemophilia When per manent survival of homografts is required in extensively burned patients who cannot provide their own skin for autografting, Toolan's recent work (202) suggests that fetal skin homografts taken from stillborn fetuses in the first trimester of pregnancy can be used with the hope of long term or even permanent survival. Other indica tions for homograft skin and membranes have been discussed in previous sections (see [21] and [22])

The innumerable uses of skin autografts have been described in many excellent textbooks on plastic and reconstructive surgery (2, 3 5 6) Some of the more difficult clinical problems that are lending themselves to skin autografting procedures are the reconstruction of the esoph agus by a tube lined with autologous skin (250) free skin grafting in the sinus, oral and pharyngeal areas in radical surgery of the head and neck for cancer (251) reconstruction of the perineal urethra with free full thickness skin grafts from the prepuce (252), construction of the vagina in congenital absence of this structure by free skin autografting (253) etc The reader is referred to the textbooks mentioned above for the actual techniques of skin grafting their daily indications pre- and postoperative care, etc and to the present author's forthcoming book on this subject.

This account of the transplantation of skin is fittingly concluded with an affirmation of the hope and confidence marking all effort in this field. To this purpose the words of the author's friend Dr Michael Woodruff are well suited (254)

"What shall we say of the prospects of ob taining permanent survival of homotransplants of skin, kidney and of endocrine tissues? The problem to be solved is complex and difficult but personally I believe that it will one day be solved Lest this optimism seems fatuous let me end by quoting the words of a distinguished American surgeon Dr Harvey B Stone Discuss ing this problem and the search for a solution in his presidential address to the American Surgical Association he said "Perhaps such a purpose today may seem visionary but let us not forget that often in the story of mankind the visionary of today has proved in some distant tomorrow to be the man of vision

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PART III

# *Cornea*

CHAPTER 4

# *Transplantation of Cornea*

RAMON CASTROVIEJO

## I ANATOMY, PHYSIOLOGY, AND PATHOLOGY OF CORNEA

The cornea is the clear, transparent, anterior portion of the eyeball and in general shape resembles a watch glass. From the front it appears to be slightly wider in its horizontal than in its vertical diameter because the conjunctiva and sclera overlap it more above and below than at the sides From the back since no such overlapping occurs, its round shape is apparent. The limbus at the periphery of the cornea forms a narrow transitional area between the cornea and sclera and differs structurally from both The curvature of the cornea is slightly greater than that of the sclera.

In front the cornea measures approximately 12 mm in the horizontal meridian and 11 mm in the vertical. At the back both the horizontal and vertical meridians are about 12 mm. The edge of the cornea is about 1 mm. thick and the center slightly thinner measuring about 0.8 mm The radius of curvature is about 7.8 mm. in the central third which corresponds to the pupillary area and flattens somewhat toward the periphery The refractive index of the cornea is close to 1.376 Most of the refraction of the eye takes place in the cornea so that irregularities in its surface or variations in the curvature of the different meridians cause refractive changes which are known as astigmatism.

### STRUCTURE OF THE CORNEA

The cornea proper is composed of five layers from the outside inward 1) the epithelium 2) Bowman's membrane 3) substantia propria or stroma 4) Descemet's membrane and 5) endothelium (figure 63)

The epithelium, of ectoblastic origin consists of five or six layers of cells. The cells of the deepest layer are cylindrical, those of the next two layers are polygonal and those of the most superficial layers are flattened and very thin. As in the epidermis the epithelial cells are firmly held together by intercellular cement substance and by cell bridges. The bottom layer of epithelial cells rests loosely on the next layer Bowman's membrane, and is continued at the limbus, with some changes by the epithelium of the bulbar conjunctiva

Bowman's membrane—like the epithelium of ectoblastic origin—is a thin structureless sheet sharply distinguished from the epithelium which it supports on its anterior surface. Its posterior surface is intimately connected with the superficial lamellae of the stroma from which it differs only in homogeneity and to which it is regarded as belonging. The membrane contains many perforations through which nerves pass to the epithelium. Bowman's membrane stops at the limbus.

The substantia propria or stroma comprises about 90 per cent of the cornea and is composed of two elements lamellae and cells. The lamellae of mesoblastic origin consist of collagenous fibrils which form broad interlacing bundles extending over the entire cornea. These bundles are arranged in layers which are parallel both to each other and to the corneal surface In the flat spaces between the interlaced fibrils corneal corpuscles or fixed cells of mesoblastic origin, are permanently located. These cells are flattened so that they fit into the flat spaces between the

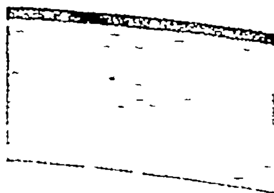


Fig. 63. Cross section through the central portion of the cornea. Microphotograph



Fig. 64. Corneal corpuscles or fixed cells. Gold impregnation. Microphotograph courtesy of Genis Galvez

lamellae and lie parallel with the surface of the cornea (figure 64). Filamentous processes from their bodies form anastomoses with adjacent cells resulting in a syncytium. In addition a few flattened migratory leukocytes known as wandering cells as distinct from the fixed corpuscles are observed in the lamellar interstices.

Descemet's membrane is a secretion from the endothelium from which it can be regenerated. It is thin and transparent and is loosely attached to the posterior layer of the stroma from which it can easily be stripped. It appears to be structureless but actually consists of two layers, a layer of elastic fibers which is in contact with the stroma and a posterior layer of mucoprotein.

The endothelium of mesoblastic origin consists of a single layer of flattened hexagonal cells which line the inner surface of Descemet's membrane. If it is damaged it is capable of regeneration.

## Nerves and Vessels of Cornea

The cornea has neither blood vessels nor lymphatics, a situation which contributes to its transparency. However a rich supply of sensory nerves derived through the ciliary nerves from the ophthalmic division of the fifth nerve enters the cornea at the limbus. Shortly after entering the cornea they lose their myelin sheaths. According to Zander and Weddell (1) the nerves enter the cornea at different levels and branch radially so that they form a plexus arranged in up to five layers between Descemet's and Bowman's membranes. The nerves which perforate Bowman's membrane to end in the epithelium are derived from the nerves in the more superficial layers of the stroma.

In addition to the sensory nerves Genis Galvez (2) has demonstrated the presence throughout the cornea of an abundant meshwork of sympathetic nerves (figure 65) which, according to this author, might have a trophic action.

## The Limbus

The limbus is the peripheral area approximately 1 mm wide which forms the transition between the cornea on one side and the conjunctiva and sclera on the other (figure 66). The corneal epithelium changes gradually at the limbus into the epithelium of the conjunctiva which is also stratified; however, the number of epithelial layers in the conjunctiva is increased to approximately ten and the cells in the basal layers are smaller and more closely packed than in the corneal epithelium. Bowman's membrane stops at the limbus and the stroma gradually changes its regular parallel cell arrangement to the more irregular opaque pattern which characterizes the connective tissue of the sclera. The anterior layers of the stroma overlap the cornea, more extensively above and below than at the sides. Descemet's membrane merges into the meshwork at the angle and the endothelium continues covering the anterior surface of the iris.

The limbus contains a dense plexus of blood vessels derived from anastomosing branches of the anterior ciliary arteries. These arteries terminate at the margin of the cornea in a series of arcades and fine capillary loops from them l



FIG 65 A sympathetic nerve fasciole in the corneal stroma. Silver impregnation method of Genis Galvez Microphotograph courtesy of Genis Galvez

formed the venous marginal plexus of the limbus which drains into the conjunctival venous system. The limbus also contains lymphatics.

The proximity of the limbus to the meshwork of trabeculae at the angle and to Schlemm's canal must be considered in interpreting certain pathologic changes which affect the healing of corneal grafts. The character of the area is especially important when grafts are large enough so that cicatrization takes place close to the limbus and to the important angle structures.



FIG 66 The transition between the cornea and the conjunctiva and sclera the limbus Microphotograph

## CHEMISTRY AND PHYSIOLOGY OF CORNEA

Chemically the cornea is composed chiefly of water which forms about 75 per cent of its material. It also comprises about five different proteins collagen mucoid elastin albumin and globulin.

Collagen is the protein found in connective tissue it is secreted by fibroblasts and plays an important role in wound healing Vitamin C deficiency delays its formation and irradiation interferes with the development of fibroblasts. If overdoses of roentgen rays or radium are given as treatment for corneal conditions such as vascularization healing of corneal incisions may be stopped indefinitely

The mucoprotein of the cornea has been identified by Meyer and Chaffee (3) as a natural monosulfuric ester of hyaluronic acid and is similar to the mucoprotein in the connective tissue of the umbilical cord Largely because of the mucoprotein if an excised cornea is immersed long enough in saline solution or water it absorbs several times its weight in water This tremendous ability to absorb water is a very important factor in corneal swelling and in the preservation of donor material. In addition to water and proteins the cornea contains lipids ascorbic acid riboflavin and various salts. Riboflavin deficiency tends to promote corneal vascularization

## HEALING OF CORNEAL WOUNDS

The study of corneal wound healing is especially important in relation to corneal transplantation. The subject is further discussed in relation to corneal wound healing in experimental animal If a limited area of the epithelium is lost from the cornea it is completely replaced from the surrounding epithelium If the entire cornea is denuded of epithelium it is replaced from conjunctival epithelium. If a wound extends deeper into the substance of the cornea Bowman's membrane and the external layers of the stroma retract, causing the wound to gape Bowman's membrane does not regenerate but epithelium covers the external gap which is

eventually filled in by fibroblastic proliferation. If the wound penetrates into the anterior chamber Descemet membrane which is elastic retract leaving a posterior gap which i filled in anteriorly by fibrinous coagulum and posteriorly by endothelial cell. The fibrinous coagulum is replaced within a few days by fibroblasts and within a few months the membrane regenerates from the endothelium.

Some antiseptics antibiotics anesthetics and also cortisone have been observed to delay corneal wound healing. Certain protein amino-acid and vitamin C and A deficiency may also delay wound healing.

Space does not permit discussion of other physiologic characteristics of the cornea such as metabolism permeability turgescence and sensibilities. These subjects are discussed in detail in specialized textbooks (4)

## CORNEAL TRANSPLANTATION

### Transparency of Cornea

Restoration of crystal clear transparency and normal refractive mechanism of the transparent cornea are the most important considerations in keratoplasty therefore the transparency itself requires some discussion. Transparency probably depends upon a combination of the following factors 1) the regular arrangement of the corneal coverings 2) the absence of blood vessels and lymphatics 3) the fairly low level of hydration in corneal tissue 4) the maintenance of intraocular pressure within certain limits 5) the extremely smooth surface of the epithelium which when it is wet with tears gives the cornea a highly polished surface 6) the presence of the proper quantity of chemically normal tears in contact with the corneal surface 7) the trophic influence of sensory fibers of the fifth cranial nerve section of the ophthalmic division of this nerve causes neuroparalytic keratitis) 8) various other factors such as the presence of mucoid which is found in large quantities in the cornea compared with the very small amounts in the sclera and the possible existence of some enzyme necessary to maintenance of transparency

The cornea is therefore the crystal clear tissue which covers the anterior chamber of the eye. Since it serves as a window through which light rays must travel on their way to the retina its transparency is essential to vision. Since it is one of the most important refractive mechanism of the eye regularity of corneal curvature also plays an important part in vision. In the absence of other ocular pathology so long as the cornea remain transparent and its curvature remains within fairly normal limits visual function is assured. But the moment the cornea becomes defective in either curvature or transparency visual function diminishes and diminishes in direct proportion to the degree of the defect in some cases down to mere perception of light

Corneal opacity dense enough to make the eye useless may follow congenital defect dystrophies deformities inflammations nutritional disturbances vitamin deficiencies ocular manifestations of general disease section or irritation of the fifth nerve or injury Detailed discussion of these or other causes of corneal opacities is beyond the scope of this chapter the reader is referred to the recent book The Cornea by Charles I Thomas (5)

If corneal changes cannot be reversed by conservative measures operation alone can restore transparency or normal form to the structure If the opacity can be removed by simply excising a portion of the cornea the operation is called keratectomy If the excised tissue is replaced by other corneal tissue from the same or other individual the operation is known as keratoplasty or corneal transplantation

### Keratoplasty

Keratoplasty or corneal transplantation is the operation which replaces with clear normal tissue corneal tissue which disease or deformity has rendered so opaque as to interfere seriously with vision The operation mentioned for the first time nearly 150 years ago was not successfully performed in man until Zirm (6) reported his case in 1906.

### Sources of Donor Material

Grafts may be obtained from three sources autotransplants obtained from the same individual homotransplants obtained from another individual of the same species heterotransplant obtained from an individual of another species

### *Types of Keratoplasty*

1) Circumscribed or partial, lamellar keratoplasty consists of replacing a circumscribed

area of cornea including approximately from one-half to two-thirds of its thickness, by healthy cornea of the same extent and thickness

2) Total lamellar keratoplasty consists of replacing the entire cornea including approximately from one-half to two-thirds of its thickness, with normal cornea of the same extent and thickness.

3) Circumscribed, or partial penetrating keratoplasty consists of replacing a limited area of the cornea including its entire thickness with healthy cornea of the same size and thickness.

4) Total penetrating keratoplasty consists of replacing an entire cornea which is badly scarred with clear cornea of the same size and thickness

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## II CORNEAL TRANSPLANTATION IN ANIMALS

The idea of corneal transplantation was advanced by Himly (1) in 1813 but was first actually reported by Reisinger (2) in 1824 A complete review of corneal transplantation in animals is not possible in this chapter but work of those whose original contributions have most strongly influenced the development of this branch of surgery will be briefly mentioned

### LAMELLAR KERATOPLASTY

Konigshofer (3) in 1830 performed lamellar homologous and heterologous keratoplasties in animals. He used a double bladed knife to outline both the graft and the host window so that both would be of the same size and shape. In 1840 Mühlbauer (4) performed initial animal experiments in reparative keratoplasty dissecting out grafts and replacing them in the original eye. Following concepts developed by Walther (5) he improved his methods and carried out a few heterotransplants, grafting animal corneas in human eyes. These grafts were in the form of equilateral triangles they extended through two-thirds of the thickness of the cornea, leaving Descemet s membrane intact (partial lamellar keratoplasty) The grafts were held in place by edge-to-edge sutures Leukomas formed after the grafts healed and some bulged permanently

In 1877 Dürr (6) introduced another form of partial lamellar keratoplasty placing rabbit corneas in human eyes The corneal portion of his grafts was triangular, 5 to 8 mm. long by 5 to 6 mm. wide and included a piece of conjunctiva which was placed at the periphery and fastened in place by sutures passed through the conjunctival flap and the surrounding conjunctiva. The epithelium became so opaque on the second day that the iris could not be seen. A few days later the grafts vascularized, but some finally cleared

In 1910 Löhlein (7 8, 9 10 11) developed still another technique of partial lamellar keratoplasty which he first used successfully in animals and them applied to human beings Using parallel knives he made two incisions 4 mm. apart extending from one limbus to the other and including at each end a conjunctival flap Only the outer two-thirds of the cornea was dissected. This rectangular strip of corneal tissue with the conjunctival flaps was transplanted to the recipient s eye which had been prepared by a similar dissection The grafts healed without much reaction but every graft became temporarily vascularized clearing later When successful transplants in rabbit s eyes were microscopically examined a year after operation Descemet s membrane and the posterior corneal lamellae were normal in structure Blood vessels had appeared in the region corresponding to the base of cicatrization and the lamellae differed from those in the host cornea in two ways they were wavy and the number of nuclei in the corneal corpuscles had increased

During the next two years Magitot (12 13 14) reported animal experiments using von Hippel's technique (see chapter 5) for partial lamellar keratoplasty. He also developed a method of rectangular grafting of his own. Perhaps his most important contribution was his work on preserved grafts. He demonstrated that corneal tissue could be preserved transparent and viable outside the body for fourteen days if it was immersed in hemolyzed serum of the same species of animal and kept at 5 to 8°C. Magitot felt that lamellar grafts were superior to penetrating ones and believed that both autoplastic and homoplastic transplantation could be successful.

Walker (15) in 1917 dissected a Thiersch graft from the superficial layers of the animal's cornea using a Graefe knife and covered the graft for 48 hours with a conjunctival flap. Walker tried his method later on the eyes of three patients with leukoma; although the transplants became opaque, they were not so dense as the original diseased cornea.

## PARTIAL PENETRATING KERATOPLASTY

Reisinger (2) in 1824 incised through the lower half of the rabbit's cornea close to the limbus using a cataract knife and held the flap with forceps while he dissected the upper part of the graft with scissors. The transplant was sutured to the host eye at the edges. All grafts became opaque, probably due to trauma by instruments and sutures, but perhaps because the irregular edges of the graft could not be smoothly fitted into the recipient's eye. Himly (1) in 1813 stated that he had suggested keratoplasty in 1813 and that his friend Reisinger described the method as his own. Himly seems, however, never to have used the method himself.

The trephine was introduced in 1840 by Steinberg (16), who used it for cutting both the grafts and the host wound of rabbits' eyes in studies of partial penetrating keratoplasty. The transplants were sutured in place with direct sutures. The experiments were not successful and the only graft which remained in place became opaque.

Strauch (17) used parallel cataract knives and scissors in 1840 for partial penetrating keratoplasty in animals. He introduced a thread into the anterior chamber to keep the globe in place during dissection and used it later to fasten the graft in place, beside suturing the graft directly to the recipient's cornea.

Markus (18) in 1841 modified Reisinger's procedure, trying to reduce the trauma to the graft by not holding it with forceps. He stated the following principles of technique: 1) the graft must be of the same shape as the defect; 2) the transplant must be transferred as rapidly as possible from the living animal to man; 3) the graft must be carefully held so that it would not be traumatized; 4) the graft must not protrude or bulge when it is placed in the recipient's eye.

Power (19) in 1872 performed a few experiments in reparatory keratectomy in glaucomatous eyes, but when he tried to carry out keratoplasties in animals or man his grafts opacified.

Ebeling and Carrel (20) in 1921 used a cataract knife to make steps on the edges of rectangular grafts to prevent them from falling into the anterior chamber. The transplants were held with forceps while they were placed and were fixed in position with six sutures. Ebeling and Carrel used no dressings but instilled olive oil into the eye after operation. Only one graft of the five placed in cats' eyes remained clear. Majewski (21 22) developed a stepped graft by using von Hippel's 4 mm. trephine to cut the superficial layers and a second trephine 3.5 mm. in diameter to cut the deeper layers of the cornea.

Filatov (23 24) in 1928 used a modification of von Hippel's method, introducing a spatula into the anterior chamber in order to separate the cornea from the lens and thus prevent injury to the deeper structures during trephination. Filatov fastened his grafts in place by means of a conjunctival flap placed with the epithelial surface against the graft.

J. W. T. Thomas (25–27), beginning in 1930, performed a series of animal studies using different techniques and differently shaped grafts. In two series of animals the transplants were rectangular, the technique differing only in that sutures were used to hold the graft in place in one series and not in the other. Of the sutured grafts 56 per cent healed, and of the unsutured 57 per cent. In another series of studies Thomas used von Hippel's trephine in some animals, cutting the entire graft with the trephine, and in others using it only to cut the superficial layers, cutting the inner layers in a shelving manner with scissors to prevent the transplant from falling into the anterior chamber.

Thomas used a slightly smaller trephine for cutting the graft than for cutting the window in the eye of the host. All transplants were fixed by means of crossed sutures inserted into the superficial layers of the surrounding host cornea. In Thomas first report, five of the total of 02 grafts remained transparent.

Castroviejo (28) in 1931 successfully placed rectangular partially penetrating homografts in animal eyes preparing both the graft and the recipient window with a double bladed knife and scissors, and fastening the transplants in place with conjunctival flaps. Three years later (29) he reported successful homografts using the same technique in rabbits whose corneas had been rendered opaque by lime burns.

During the next decade preservation of donor material was studied in animals, especially by Scherschewskaya (30) (1940) Castroviejo (31) (1941) and Paufique Sourdille and Offret (32) (1948) Scherschewskaya used devitalized rabbit cornea preserved in formalin and reported that twelve grafts had healed of which three remained transparent. Paufique and his associates preserved rabbits corneas in alcohol, but the grafts did not cicatrize and all became opaque. Castroviejo performed experiments with rabbit and human corneas to determine whether or not it was practical to use grafts preserved in formalin and obtained no transparent grafts using this method. He then carried out further studies to learn the best methods for preserving donor material from cadavers and also how soon after death eyes had to be removed so that the tissues would be healthy His conclusions were 1) it is not advisable to use grafts obtained from cadaver eyes more than 24 hours after death because changes in the eye after this period may render the graft nebulous or opaque 2) the eye can be preserved in a more normal condition for a longer time in a moist chamber than when it is immersed in various solutions

## MICROSCOPIC STUDY OF CORNEAL GRAFTS IN ANIMALS

Considerable study of corneal grafts has been carried out to determine whether the graft is replaced by elements from the host or whether it preserves its own individuality The question is still controversial. Early investigators who believed in the replacement theory included Marchand (33 34) who in 1898 and 1890 studied homotransplants and autotransplants in rabbits Salzer (35 36 37) who in 1900 and 1921 found that autotransplants were successful homotransplants only exceptionally successful and heterotransplants never so and Bonnefon and Lacoste (38 39) The latter after performing some 200 partial lamellar keratoplasties in animals, concluded that only the epithelium the lamellae of the stroma and a few corpuscles of the original graft were preserved, but that the rest of the graft was replaced from the host.

On the other hand microscopic observations of partial penetrating keratoplasties on rabbits eyes led Castroviejo (40) in 1932 to believe that transplants which remained entirely transparent were normal in structure. He observed no evidence that tissue from the host had replaced the graft tissue at any point, except in complicated cases in which the cornea had been rendered cloudy or opaque because of infiltration vascularization or fibroblastic proliferation over the surface of the entire graft or over a part of it *The epithelium endothelium and Descemet's membrane if partly or totally absent in the donor graft, can be regenerated from the host but if these elements are intact in the graft they preserve their individuality and are not replaced by elements from the host cornea* Five years later (41) fuller studies of partial penetrating homografts in rabbits dogs, monkeys and human beings corroborated these earlier observations In addition special investigations indicated that heterografts almost invariably became opaque and caused severe reaction in the host eye. On the other hand, comparison of the results of autografts, homografts and heterografts showed that autografts and homografts caused little reaction, and when properly done resulted in excellent vision

Basing his conclusions on study of several body tissues, Peer (42) has advocated the cell survival theory He writes "In humans the cells in free autogenous grafts tend to survive and retain their normal structure when transplanted as complete cell entities in favorable transplantation sites When cells in free grafts fail to survive the graft is replaced by connective tissue but this replacement is not a duplicate of the original graft." After careful study of the behavior of ten commonly used free tissue grafts in humans the author (Peer) concluded that the great majority of these grafts probably survive and are not replaced by host tissue cells More limited studies however Peer finds do not

suggest that this conclusion is entirely applicable to homotransplants, which he believes tend to be gradually and slowly replaced from the host. One exception to this replacement of homogenous grafts by the cells of the host is corneal tissue. Peer says: "Under favorable conditions many fresh homogenous corneal grafts remain clear and it is generally accepted that the cells remain viable and retain their characteristic clear collagenous matrix."

The cell survival theory is steadily gaining adherents. At present most surgeons who are experienced in corneal transplantation believe that a transparent graft retains its own individuality.

## CONCLUSIONS

After the initial work on animal experimentation, which covered over one hundred years, contributions became so numerous that it is not possible to discuss even the most important. From the analyses presented, the results of keratoplasty in animals may be summarized as follows:

1) Corneal grafts placed in animals are practicable when suitable techniques are used.

2) Only autogenous and homogenous grafts remain transparent in the host's eye. Heterologous grafts remain transparent only under exceptional circumstances [illegible] which are difficult to explain or to duplicate experimentally.

3) Eyes obtained [illegible] animals must be promptly enucleated [illegible] hours catalytic changes ordinarily [illegible] corneas useless for grafting.

4) Donor eyes [illegible] preserved for several days at a temperature [illegible] between 2° and 4°C. Grafts immersed in [illegible] solution become swollen, cloudy and thick so that they are unfit for use as grafts.

5) Preserved material is suitable for grafting as long as it is clinically transparent. Keratoplasty should be carried out as soon as possible after enucleating; the longer the delay, the poorer the chances of satisfactory results.

6) Corneas preserved in formalin are not suitable for keratoplasty.

7) There seems to be abundant evidence that when the graft remains transparent it retains its individuality and preserves its own cellular elements.

Although there is no convincing evidence that the cells of the graft are replaced from the host, nonetheless certain damage to the transplant may be repaired from the recipient's cornea. Damaged epithelial cells, for example, may be regenerated from host epithelium, and injured endothelium or Descemet's membrane may be replaced from host structures, resulting in a new unclouded lining to the cornea. Damage to the stroma may be repaired, but at the expense of permanent clouding or opacity: the repaired stroma contains abnormal elements which do not appear in the host stroma. It seems probable, therefore, that reports indicating that the graft is gradually replaced from the host have been founded on examination of abnormal elements in opaque grafts which are not found in transparent grafts, and that these abnormal elements have misled investigators to believe that all graft tissue, not only injured tissue, is replaced from the host.

The only element in the graft which does die and must be replaced from the host is the nerve tissue. Much more investigation of a highly technical nature is needed to learn whether the cornea contains sensory nerves only or whether sympathetic nerve fibers are also present. Recent reports suggest that the normal cornea has not only sensory but also sympathetic fibers.

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# *Transplantation of Cornea (Continued)*

RAMON CASTROVIEJO

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## I CORNEAL TRANSPLANTATION IN MAN

The earliest attempts at keratoplasty in human eyes failed chiefly because the pioneer surgeons acted on the belief that animal corneas could be transplanted into the eyes of man. Thomé (1) in 1834 removed a portion of the cornea from the donor with a spade-shaped knife and fixed it in the host window with a single suture. The grafts made in this way were not successful but stimulated the interest of Bigger (2) The latter used a Beer cataract knife to dissect portions of cornea from rabbits and fastened them in the recipient window with direct sutures. Bigger thought that his experiments were successful enough to prove that keratoplasty could be carried out in man and recommended the pig as the most suitable source for donor material. In 1844 Kissam (3) reported grafting a human eye with a young pig's cornea. The graft took but became opaque Still another early unsuccessful attempt to graft animal corneas into human eyes this time using sheep cornea, was reported in the same year by Wutzer (4) Wolfe (5) in 1880 in[illegible]ured a technique for partial penetrating [illegible] similar to that adopted later by Löhlein [illegible] lamellar grafts. One of his patients was able to count fingers and distinguish features two weeks after operation.

Apparently the first homotransplant was transplanted by Sellerbeck (6) in 1878 using a graft from a fetus. Unfortunately the operation was not successful

Of all the men in this early period however the one who probably gave the greatest impetus to keratoplasty was von Hippel (7 8) Like Steinberg over thirty years before von Hippel in 1877 and 1878 used a trephine to excise the graft and to outline the host window His trephines, from 4 to 5 mm. in diameter were provided [illegible] sheathlike guards by means of which the dep[th] of the cut could be regulated. Gentle pressure [on] a push button started a watch-like motor wh[ich] actuated the blade. Although the first operati[on] using this trephine—a partial penetrating ke[ra]toplasty—failed, the report of the techniq[ue] stimulated wide interest.

Power (9) in the same year as Sellerbeck's re[port], advocated the use of homologous mater[ial] not because of biologic intolerance, however b[ut] because such material was easier to fit. Power al[so] recommended using transplantation of large disk[s] or of the whole cornea with a small ring of co[n]junctiva which was fastened to the host conjun[c]tiva with marginal sutures. Von Hippel defende[d] small grafts in preference to the large ones pr[o]posed by Power and believed on the basis of h[is] experience that heterologous donor material w[as] superior to homogenous grafting. In 1886 an[d] 1887 he had reported a case in which the pa[tient's] vision had been considerably improved b[y] a full thickness graft from a rabbit cornea place[d] in the patient's eye as a lamellar transplant, an[d] he had observed a case of panophthalmitis fol[-]lowing a homograft

In 1888 von Hippel (10) reported his metho[d] of lamellar keratoplasty Using his trephine b[e] removed disks 4 to 5 mm. in diameter extending through the outer opacified layers of the corne[a] and leaving clear layers of stroma, Descemet membrane and endothelium intact. The excise[d] areas were replaced by disks taken from the ful[l] thickness of dog cornea. Most of the transplant[s] adhered in about ten minutes and remained

transparent to some degree. As a result of these studies von Hippel believed that the optimal size and form of corneal grafts had been established. Lamellar keratoplasty, moreover, he stated to be easier than penetrating keratoplasty and offered certain other advantages. It did not expose the patient's eye to possible loss of vitreous fluid; it avoided displacement of inner ocular structures; healing was better since the base and surrounding host tissue remained intact, permitting rapid adhesion. In von Hippel's experience, healing was complete at the end of three weeks, but a nebulous infiltration remained at the base of cicatrization. The final graft, however, was clearer than the original leukoma.

Von Hippel's techniques have formed the basis for most keratoplasty since his time. Variations are limited largely to small differences in the manner of fixing the graft or to improvements in methods of dissection. Fuchs (11) and Smead reported that they had achieved improved vision using von Hippel's methods, and Fuchs in 1894 reported on 30 patients who had had corneal homografts done in this way. Two patients treated by partial penetrating grafts had somewhat improved vision.

Zirm (12, 13) in 1906 operated upon the eye of a patient whose cornea had become leukomatous as a result of a lime burn, and used von Hippel's trephine for the procedure, fixing the graft by means of crossed sutures inserted into the host's conjunctiva at opposite sides of the graft. One year after operation the patient's vision was 6/36, whereas before transplantation he had been able only to distinguish hand movements. Zirm concluded: 1) Only eyes of young persons are suitable sources for grafts; 2) all operations should be done with von Hippel's trephine; 3) the margins of the graft should match the surrounding tissue; 4) eserin should be instilled if the anterior chamber is present; 5) deep narcosis and strict asepsis are essential, but no antiseptics should be employed; 6) the donor disk should be protected between pieces of gauze moistened with sodium chloride and warmed with steam; 7) grafts should be held in place by two sutures at right angles crossing over the center of the graft and inserted into the bulbar conjunctiva; and 8) keratoplasty should be performed only in cases of central corneal scarring.

Autoplasty was performed in 1908 by Plange (14), who carried out a superficial keratoplasty in a cornea with leukoma caused by lime burn and replaced the tissue with a lamellar graft from the same patient's other eye, which was blind. He used a Graefe knife for the dissection and inserted the graft like a watch crystal, leaving a transparent area of 5 by 7 mm. through which the pupil was visible. At the end of a few weeks the transplant had vascularized, but it cleared later so that the patient could count fingers at 4 to 5 m. and remained clear for five years.

Calderaro (15) of the Cirincione Clinic in 1908 reported two cases in which penetrating keratoplasty had been done according to von Hippel's methods. Final vision was 1/20 in one patient and 1/10 in the other. Löhlein in 1910 reported successful lamellar keratoplasties in human eyes using the techniques on which he had earlier reported in animal studies (see chapter 4, I.)

Morax (16) and Magitot (17) presented autografts done by transposing a lamellar disk of opaque cornea in the pupillary area and a clear disk from the peripheral region of the same eye. The method was of course useful only when central corneal opacities were of limited size. Kraupa (18) the next year reported a variation on the transposition concept. With a trephine he cut a full thickness disk which included both dense central cornea and clear peripheral tissue. The disk was then rotated 180° so that the position of the clear and opaque areas was reversed and the clear portion lay over the pupil. Years later Gradle developed a similar technique, but applied it to lamellar grafts. In 1913 Magitot (19) used the technique which he had developed in animals to place a rectangular partially penetrating graft in a human eye.

The reports from Elschnig's clinic at the Prague school for the first time made it possible to study a large enough group of operations so that valuable conclusions could be drawn. Ascher (20, 21) in 1919 and 1922 wrote comprehensive reports of operative results. Elschnig (22, 23) in 1920 and 1922, Gradle (24) in 1921, Stanka (25) in 1927, Liebsch (26) in 1930, and Elschnig (27) again in 1930 presented cases. At this clinic von Hippel's method of partial penetrating keratoplasty was followed with this modification: instead of the loose graft, it was fastened in place with bridging sutures formed by inserting the thread through the conjunctiva near the upper limbus, passing it over the graft, and tying it through the conjunctiva on the opposite side. Transplants remained clear in 22 per cent of 174 cases reported, but of the cases with interstitial

keratitis which was considered the most favorable indication for keratoplasty 73 per cent of the operations were successful. Elschnig stated that donor material could be taken from either young or old eyes and that so long as the cornea was normal the condition of the rest of the anterior segment was immaterial. He also felt that it was unimportant whether or not the patient had glaucoma or hypotension. Elschnig found no connection between blood groups and transparency or opacity

Filatov (28–32) in 1920 Thomas (33–37) in 1930 and Castroviejo (38–48) in 1931 reported first animal studies and later clinical experiences with partial penetrating keratoplasty Filatov and Thomas used a modification of von Hippel's trephine technique, and Castroviejo a rectangular graft cut with a double bladed knife and scissors. Since then the reports on keratoplasty both experimental and clinical have been so numerous that it is impossible to mention even a small proportion of the valuable contributions. However the work of Nižetić (49 50) Nicolato (51 52) Franceschetti (53–56) Friede (57–61) Fine (62 63) Paton (64) MacLean (65) Maumenee (66 67) Arruga (68) the Barraquer (69 70) Venco (71) Rycroft (72) and Thomas (72A) deserve special mention The work of such men has confirmed Elschnig's experience and shows that autografts and homografts are feasible and in properly selected cases give a high percentage of favorable results.

The book by Paufique, Sourdille and Offret (73) *Les Greffes de la Cornée* is one of the most important contributions. Following the tradition of Morax and Magitot these investigators demonstrated anew with successful cases the usefulness of lamellar grafts which under the influence of Elschnig had been largely abandoned. Paufique (74) described in detail a technique of partial lamellar keratoplasty and Sourdille (75 76) a method of total lamellar grafting

Up to this time partial and total lamellar and partial penetrating keratoplasty were the only techniques which had been established. Total penetrating keratoplasty had been tried by Wagemann (77) in 1888 by Schimanowski (78) in 1912 by Burke (79) in 1921 by Filatov (30) and Elschnig (22) in 1922 by Key (80 81) in 1930 and by Friede (59) in 1936 but the operations had not been successful the grafts became opaque, and the eyes were eventually lost because of secondary glaucoma or phthisis bulbi.

Castroviejo (47 48) in 1950 reported the first successful cases of total penetrating grafts, all performed in unfavorable cases which did not suggest that visual improvement would follow other types of operation. Of 21 grafts, 13 either remained clear or were slightly cloudy with visual improvement six to ten months after operation. After a longer period of observation only 25 per cent remained clear enough to improve vision. One graft has remained clear for over seven years.

## DONOR MATERIAL

Materials used for corneal transplantation must be in an excellent state of preservation. If a corneal graft is placed to restore vision, it not only must cicatrize but must remain transparent. The slightest opacification in the graft is likely to reduce vision enough to defeat the purpose of th operation

In 1941 the author (39) carried out some experiments with animal and human corneas to find ou how long after death enucleation could be post poned and how long after enucleation from cadavers the cornea could be kept viable and i condition for successful grafting Animal studies indicated that if the cadaver was refrigerated the eye should be enucleated within 24 (preferably 12) hours in order to prevent cadaveric changes in the cornea. As preservatives, he found little difference between auto- homo- and heterosernms, Ringer's solution and normal saline solution if the eyes were kept at a temperature of 2° to 4°C. However further study showed that the eye could be kept in better condition for a longer time in a moist chamber than when it was immersed in any solution. These studies were then extended to ascertain how long human eyes could be preserved without undergoing cadaveric changes. Eyes enucleated from stillborn infants and from cadavers of older infants and adults were studied. Eyes preserved in normal saline and in a moist chamber behaved exactly as had animal eyes, except that corneas from stillborn infants became hazy and swollen when they were immersed in solutions much earlier than eyes of older infants or adults. It was also observed that cadaver eyes became soft by the second day and that corneal wrinkles appeared after two or three days preservation. Hazy streaks which developed among the wrinkles made the eyes unsuitable for corneal grafting carried out to improve vision

As a result of these investigations, certain procedures for obtaining and preserving donor ma

terial have evolved, which have been generally adopted by organizations and eye banks supplying eyes for corneal transplantation Eyes of stillborn infants children or adults are enucleated as promptly as possible after death. They are thoroughly washed with normal saline or a solution containing an antiseptic or antibiotic, and placed on a piece of saline-moistened gauze in the bottom of a large test tube or specimen bottle care being taken to see that the cornea does not come in contact with the moist gauze if this should occur the tissues may become hydrated. The container is tightly closed and placed in a refrigerator at a temperature of 2° to 3°C The donor material should be used as promptly as possible either on the day of enucleation or on the next day Although the corneas of some preserved eyes remain clear for three days it is advisable to use them within 24 hours if possible in order to minimize changes which may jeopardize the success of the operation If the donor graft is obtained from an eye enucleated from a living person because of some condition which does not affect the cornea the procedure varies somewhat with circumstances. If the donor graft is to be used very shortly the eye may be immersed in normal saline until the disk is dissected out. If keratoplasty is not to be performed for several hours the eye should be placed in the moist chamber If the keratoplasty is to be performed in some city other than that in which the enucleation is done, the glass bottle containing the eye is placed in a metal thermos and sent by special delivery if this method insures prompt enough delivery otherwise it is sent air mail by special delivery

In an effort to prolong the short period that eyes may be preserved at present various other methods of preservation have been suggested Bürki (83) in 1948 advocated using liquid paraffin, a tissue storage method previously used by Carrel. Burki has presented evidence that eyes preserved in this way remain viable for several weeks Rycroft (72) in 1950 reported that eyes removed within ten hours after death and stored in liquid paraffin provided consistently successful lamellar and full thickness grafts up to three weeks after enucleation

Grafts obtained from donor material frozen at very low temperatures have not been successful to date However some evidence is accumulating that eyes pretreated with dilute glycerol solution before freezing may provide satisfactory graft material after relatively long periods of storage Eastcott and associates (83) in 1954 treated corneas of various thicknesses with 15 per cent glycerol in Ringer's solution for one hour and then froze them for storage at −74 C When they were wanted for use they thawed rapidly Lamellar grafts made from such material after several weeks storage were satisfactory and their behavior did not differ after operation from that of grafts preserved in the usual way Results of full thickness grafts were not however so satisfactory as results of lamellar grafts.

King (84) has recently reported successful lamellar grafts by using donor cornea dehydrated in 95 per cent sterile glycerine sealed in a vacuum, and stored indefinitely without refrigeration So far the use of such material for penetrating grafts has not been adequately investigated

The problem of donor material has been solved in some countries by legislation permitting removal of eyes from unclaimed cadavers solely for the purpose of corneal transplantation but in the United States the supply of donor material is still insufficient so that the surgeons have to place their patients on waiting lists During the past few years the situation has improved because members of various organizations have pledged their eyes to be used after death for corneal transplantation Furthermore, the establishment of eye banks has increased the supply and has made distribution of the material more efficient. However as an increasing number of ophthalmologists become proficient in the techniques of keratoplasty the demand will not be met unless legislation such as has been passed in some other countries is also passed in the United States or unless some of the newer methods of preservation prove successful

## MICROSCOPIC STUDIES OF GRAFTS IN MAN

After reviewing studies of grafts by other authors such as Thomas (34 36 37) von Fieandt (85) Filatov (29 32) Franceschetti (55 56) Babel (86–89) and Valcarce (90) and carrying out studies of his own Offret (73 91) wrote

> First of all the penetrating graft is shown in all cases of successful transplantation as a fragment of normal cornea in the center of pathological cornea The graft is separated from the neighboring region by a scarred area which subsides in time but may still be visible after six years It is in this area that histological changes are produced

changes in grafts are insignificant or in later stages invisible

Thus according to Offret, the region in a graft which demands attention is the limiting ring corresponding with the scar at the point of union of the graft with the host tissue. Microscopically this ring appears the same as the scar of a corneal wound. The width of the ring varies according to the manner of healing if the wound heals smoothly and uneventfully within a few months after operation it looks like a thin whitish-grey line which gradually fades so that after a year or two it is almost invisible except by microscopic examination However if the edges are in poor apposition or if healing was for some other reason difficult, excessive proliferation of fibroblasts may form a linear corneal leukoma at the ring the width of which varies according to the amount of scar formation needed to repair the defective union.

From the biologic viewpoint, this ring of scar tissue is extremely important (91) since exchanges between the graft and the host must pass through it. If Offret's view is correct, a normal ring reestablishes growth and the resultant continuous tissue insures nutrition of the graft and maintenance of transparency On the other hand, if the ring is abnormal, especially if the abnormality is caused by displacement of the graft transparency is impaired. The limiting ring in a transparent graft also has a curious ability to select the elements which cross it. For instance, it is an impassable barrier to corneal vessels and very few nerves cross a thick ring

## Innervation of Graft

Franceschetti (55) Babel (88 89) and Valcarce (90) have found by microscopic examination that when grafts become opaque they usually are abundantly innervated and that nerves usually follow blood vessels. Franceschetti and Babel examined a graft which had remained clear for six years and found a large number of nerves in the host cornea but none in the graft. Babel found a few nerves in the deeper layers of a clear graft but only rare fibers of a questionable nature in the superficial layers of the stroma. On the other hand Valcarce found abundant nerves in the central portion of a graft which had remained clear for three years and found them not only in the deeper layers of the stroma but also in the subepithelial region.

Moss (93) in 1940 studied the regeneration of corneal nerves clinically by comparing the sensitivity of the graft with that of surrounding tissue. The eyes of 26 patients 24 with partial penetrating grafts and 2 with lamellar grafts, were used for the investigation. Their ages varied from 14 to 61 years, and the operations had been performed from 2 weeks to 10 years earlier Twenty four grafts were clear and 2 were hazy In 7 instances responses to sensitivity tests indicated completely normal sensory innervation in 4 sensitivity had partly returned and in 14 there was no response. Results were uncertain in one case.

The earliest evidence of returning sensitivity was observed in a graft 2¾ months old but responses were negligible in all other grafts less than 4 months old. Sensitivity had completely returned in one graft 10 months after operation, and the oldest graft showing evidence of returning sensitivity was 3½ years old. Moss concluded 1) Functional sensory reinnervation does occur on the surface of corneal transplants. 2) The return of sensory innervation is not related to the age of the graft, the age of the patient, the corneal condition which indicated grafting, the shape of the graft, or its clarity 3) Since some clear grafts remain insensitive, it seems unreasonable to assume that trophic innervation would have occurred without sensory innervation therefore no trophic innervation seems necessary for continued transparency of the graft. This study and the author's own observation suggest that if sensory innervation returns to the corneal graft, it ordinarily appears in 4 to 6 months.

## PATHOLOGY OF GRAFTS IN MAN

Most surgeons who perform keratoplasty have studied some of the pathologic changes which lead to cloudy or opaque grafts either clinically or experimentally Some of these changes are caused by postoperative complications, others are induced by the original condition which made transplantation necessary The question is too extensive to discuss fully here where we are concerned chiefly with the fundamentals of corneal grafting rather than with clinical observations. One special aspect of the problem, however deserves special attention, *viz.*, the problem of sudden pathologic change leading to cloudiness or opacity within weeks or months after an uncomplicated operation in a favorable eye, followed by an uneventful recovery with every evidence that the graft would remain permanently clear

The French school call this sudden change the Maladie du Greffon, or Disease of the Graft

The reasons for this change are still uncertain and controversial. Maumenee attributes it to donor recipient sensitization and therefore considers the opacity the result of an allergic reaction of the host to foreign proteins in the graft. This belief is based on carefully conducted homokeratoplasties in rabbits When at the end of two weeks the transplants remained clear Maumenee inserted pieces of skin from the original donors of the graft material under pocket flaps in the abdominal walls of the recipients. Whereas ordinarily one in 60 eyes would have become cloudy inclusion of the skin caused 28 of 30 eyes to become nebulous within 2 to 3 weeks. Maumenee drew the following conclusions 1) A donor-recipient sensitization is capable of producing opacification in corneal grafts in rabbits. 2) Clinically the reaction in these rabbit eyes is similar to the clouding which occurs in successful human grafts 2 or 3 weeks after successful operation. 3) The fact that supplemental skin transplantation produces clouding in corneal grafts indicates that destruction of homotransplants is the result of donor-recipient sensitization and that the allergy so produced affects more than one type of tissue from the same animal. It is true that the vascular tissue used by Maumenee did produce an allergic reaction which affected more than one type of tissue, including the cornea. The experiments might have been more pertinent to the problem of keratoplasty had avascular corneal tissue been used instead of skin

In the author s clinical experience, however the Disease of the Graft has appeared only when it has been possible to demonstrate foci of infection, especially of the teeth or upper respiratory tract, such as tonsillitis or sinusitis. Postoperative graft reactions can often be cleared by neutralizing such infections with antibiotics, antihistamines cortisone and similar drugs or foreign protein therapy and at the same time by building up the patient s resistance with high protein diet and other supportive measures. If these treatments fail, surgical removal of the source of infection may save the transparency of the graft if operation is carried out before irreversible changes occur The fact that all symptoms of corneal irritation stop when these causes are eradicated indicates that the inflammation is of bacterial origin rather than due to an allergic nonbacterial reaction Moreover patients who have been dismissed 5 to 8 weeks after operation with clear grafts and greatly improved vision frequently return a few days or weeks or months exceptionally years, after dismissal complaining that vision has suddenly diminished coincidentally with a severe head cold or sore throat. The appearance of these eyes is identical with that of eyes with Disease of the Graft. Unless irreversible changes have taken place adequate treatment of the cause of the sudden corneal reaction reduces the eye inflammation and restores transparency to the graft.

## CORNEAL WOUND HEALING

Both microscopic and clinical studies show that cicatrization of the graft is not complete until about a year after operation. The most critical period is the first few weeks especially the beginning of the third week. Until this time the graft is maintained as a tissue culture and is held loosely in contact with the host by fibrinous material Since it lies in the midst of corneal tissue and has no blood vessels, the nutrition of the graft during the period of cicatrization is precarious therefore all circumstances must be favorable if the graft is to heal in such a way that it maintains its transparency If the apposition of the edge of the graft to the recipient window for example, is defective, the result will be poor cicatrization, excessive fibroblastic proliferation, impaired nutrition and eventually a nebulous or opaque graft.

If the host is pathologically affected with acute or chronic infection, especially if the host has poor resistance, toxic substances may enter the graft. Since during the early stages of healing, exchanges between the graft and host are precarious, proteolytic ferments with histamine-like effects concentrate in the graft. The more the graft is saturated with such products, the more the *nutrition* of the graft becomes impaired and the more *necrotic* changes appear in the graft Such changes are followed by inflammatory reactions, infiltration, vascularization and fibroblastic *proliferation* and lead eventually to permanent impairment of transparency These reactions correspond with those observed in the Disease of the Graft which may therefore occur from causes other than donor recipient sensitization although protein allergy cannot be excluded as a possible cause in certain cases

Six *months after* operation gaps in Descemet s membrane have been closed by secretions from

the endothelium. Nerves have regenerated The line of cicatrization, which during the first few months formed a barrier to the entrance of nutrients has become more permeable. The exchanges between the graft and the host improve steadily as the graft becomes more permanently established as a part of the host organism. Because of this improved exchange grafts which have been cloudy may clear during the second six months. At the end of a year cicatrization is complete and the graft well rooted in the organism. A graft which remains cloudy and opaque at the end of a year is unlikely to improve. If the transplant was performed to improve vision and vision remains seriously impaired by opacity another keratoplasty is indicated. It is unwise to carry out other surgical procedures, such as cataract extraction until this six months healing period is past otherwise insult due to the second operation may cause the graft to cloud

## CONCLUSIONS

Survey of the literature on corneal transplantation leads to the following conclusions

If suitable techniques are used and cases are favorable, auto- or homokeratoplasty may lead to a high percentage of clear grafts with greatly improved vision.

Donor material must be in optimal condition if the best results are to be obtained from keratoplasty

Donor material may be preserved for a few hours immersed in an isotonic solution. If eyes are to be kept longer they should be placed in a moist chamber or in liquid paraffin, and refrigerated Studies of methods which may make it possible to preserve donor material longer than at present are encouraging and may help to increase the supply

Eye banks and other organizations which collect and distribute eyes are partially solving the increasing need for donor corneas Legislation permitting enucleation of eyes from unclaimed cadavers would help to meet the increasing demand for donor material

Most ophthalmologists tend to accept the theory that the graft survives rather than the theory that it is replaced from the host.

It is still uncertain whether opacification of the graft after a successful operation is in some cases caused by a donor-recipient sensitization or to other pathologic conditions in the host

The reasons why the graft remains transparent are still uncertain. Further investigation is needed in order to determine whether regeneration of nerves or other factors are essential to successful grafting

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## II CLINICAL USE OF CORNEAL GRAFTS

### GRAFTS FOR IMPROVEMENT OF VISION

Keratoplasty is most frequently performed to restore vision which disease or deformity has rendered insufficient for the patient's needs. Vision of 20/200 classified as industrial blindness is generally considered poor enough to indicate the operation but under some circumstances a corneal graft may be advisable even when the patient sees much better than this.

Techniques of keratoplasty vary according to the shape of the graft, its size thickness (partial or full) and the mode of fixation. Techniques improve continuously as new instruments, better suturing materials and other technical advancements become available.

#### Types of Keratoplasty

*Circumscribed or Partial Lamellar Keratoplasty*

A circumscribed area of the external lamella comprising approximately one-half to two-thirds of its thickness is replaced by transparent cornea of the same size and thickness, taken from a donor eye. In favorable cases this type of keratoplasty gives a high percentage (90 per cent) of good results.

*Total Lamellar Keratoplasty*

The external layers of the whole cornea comprising approximately from one-half to two-thirds of its thickness, are replaced with a graft of similar size and thickness from a transparent cornea. This operation is used in cases of superficial opacity extending over the entire area of the cornea. Although it is not as likely to be successful as a smaller graft it gives a fairly high percentage (60 per cent) of successful results in selected cases.

*Circumscribed or Partial Penetrating Keratoplasty*

A variable area of the full thickness of the opaque or deformed cornea is replaced by a corresponding piece of normal cornea. This is the type of operation most frequently used at present and is suitable to the greatest number of eyes affected with corneal opacities or deformities. In favorable cases it gives a high percentage (90 per cent) of successful results.

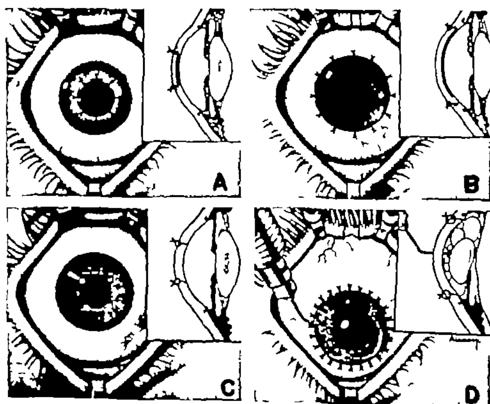



Fig 67 Types of keratoplasty A Partial lamellar B Total lamellar C Partial penetrating D Total penetrating

*Total Penetrating Keratoplasty*

The entire cornea is transplanted to replace a severely scarred cornea which is diseased or deformed over the entire or nearly the entire surface. Until a few years ago this type of keratoplasty was not reported as completely successful During the past seven years the author has reported cases of successful total penetrating keratoplasty some already under observation over seven years. Total penetrating keratoplasty *is performed only in extreme cases when other* types of operation cannot be carried out successfully It is undertaken only in eyes still preserving good perception and projection of light, and in which tension is within normal limits. Total penetrating keratoplasty results in about 25 per cent of either transparent or fairly transparent grafts with some or considerable, improvement in vision.

**Use of Different Types of Grafts**

Lamellar grafts do not ordinarily achieve as brilliant results as penetrating grafts. Vision of 20/20 or better which not infrequently follows penetrating grafts, is exceptional after lamellar grafting Unquestionably however lamellar grafts are safer because they eliminate such complications as iris incarceration or prolapse injury to the lens secondary glaucoma, or endophthalmitis *In spite of the less satisfactory results therefore* lamellar grafts are chosen when the factor of safety is exceptionally important for example in one-eyed persons and for aphakic eyes. For the *same reason they are selected for children or* other patients who are likely to jeopardize the success of the operation by unruly behavior during the postoperative period.

*Lamellar keratoplasty is also preferred when* the opacity is superficial especially if the opacity is restricted to the pupillary area and the rest of the cornea is in good condition Lamellar grafts are sometimes used when the operation is performed chiefly for cosmetic reasons. They are practical for removing superficial opacities which appear after penetrating keratoplasty Although vision may be better if a penetrating graft is used a lamellar graft benefits corneal dystrophies of the Fleischer Groenow or Haab-Dimmer types and also other dystrophies which do not involve the deeper layers of the cornea.

Unless lamellar grafts are clearly indicated by some of these considerations, penetrating grafts are preferred and are indicated in the largest proportion of cases.

**Indications for Keratoplasty**

With the knowledge available today the surgeon can predict fairly accurately the chances of a *successful* operation on a particular eye If for example the deformity or opacity is central and is surrounded by healthy tissue, the chances of success are high at the other extreme, if dense scarring covers most of the cornea, especially if vascularization has become established the chances of a successful outcome are limited. Eyes may be classified as I, favorable II moderately favorable and III unfavorable.

*Group I Favorable*

*In this group about 90* per cent of clear grafts may be expected, with final vision usually over 20/50 and not infrequently achieving 20/20 or better Favorable eyes include those with the *following conditions*

1) Central corneal opacities surrounded by healthy corneal tissue (figure 68)

2) Keratoconus, if vision cannot be improved by regular or contact lenses or if contact lenses are not tolerated If the transplant is not large enough to replace the entire conus the graft may heal unevenly with protrusion in such instances a high degree of astigmatism or myopia may cause such poor vision as to defeat the purpose of keratoplasty (figure 69)

3) Interstitial keratitis if the opacity is not too dense and extensive so that the graft will be in contact with fairly healthy corneal tissue.

4) Corneal dystrophies of the Fleischer Haab-Dimmer and Groenow types.

*Group II Moderately Favorable*

Keratoplasty can be expected to give clear *grafts, or at least considerable improvement of* vision, in about half of all eyes with the conditions mentioned below

1) Superficial opacities extending over the entire surface of the cornea *if the epithelium* appears healthy if the cornea is not superficially vascularized and if it can be determined that most of the layers of the stroma under the opacity are healthy (figure 70)

2) Tear-gas burns if there is no pannus-like superficial vascularization and only a limited area

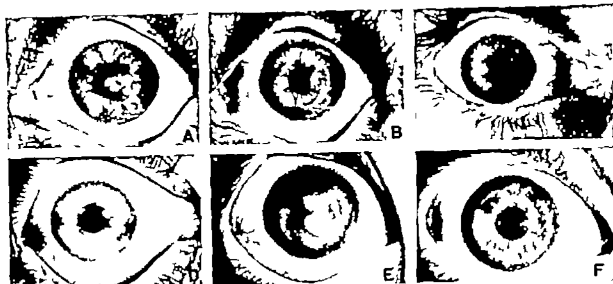



Fig 68 Keratoplasties in favorable cases A. Central superficial corneal opacity following ulcer B After partial lamellar keratoplasty C Groenow's dystrophy D After partial penetrating keratoplasty E Superficial corneal opacity sequela of recurrent keratitis F After partial lamellar keratoplasty

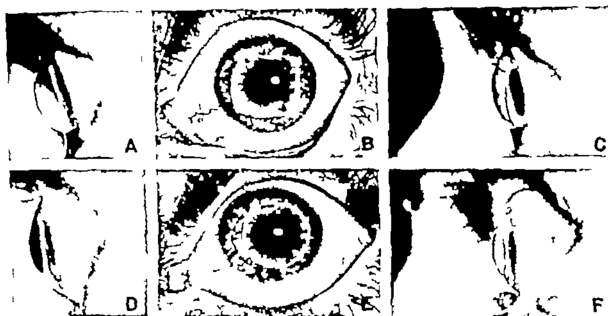



Fig 69 Keratoplasty in favorable cases A Advanced keratoconus B and C After partial penetrating square keratoplasty D Advanced keratoconus E and F After partial penetrating circular keratoplasty

of the superficial cornea is destroyed leaving enough healthy tissue to nourish the graft.

3) Adherent leukomas provided the iris is freed from the corneal scar by preliminary operation before the keratoplasty is undertaken.

4) Interstitial keratitis greater in extent and more densely opaque than in Group I but with enough permeability remaining in the stroma to indicate that a graft should remain more transparent than the original diseased cornea (figure 70)

5) Salzmann's corneal dystrophy

6) Fuchs epithelial and endothelial dystrophy, in the early stages and when only the center of the cornea is affected and the entire diseased portion can be excised (figure 71)

*Group III Unfavorable*

Keratoplasty carried out on eyes with the conditions listed in this group is unlikely to be successful However in some instances preliminary operations to be described later in this chapter

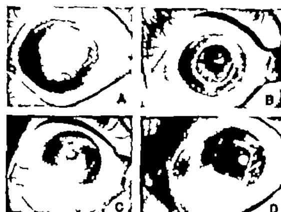



Fig 70 Keratoplasties in moderately favorable cases A Extensive superficial corneal opacity sequela of keratitis B After partial penetrating circular keratoplasty C Interstitial keratitis sequela of syphilis D After partial penetrating square keratoplasty

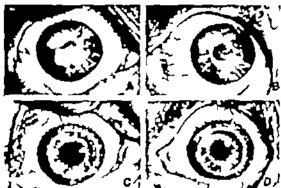



Fig 71 Keratoplasties in moderately favorable cases A and B Fuch's epithelial and endothelial dystrophy affecting only the central portion of the cornea C and D After large partial penetrating circular keratoplasty

may improve the chances of success enough to warrant grafting

1) Corneal scars which include the pupillary area and extend to the limbus. In these cases the cornea is likely to become vascularized and the graft, opaque

2) Leukomas extensive enough so that over half of the graft would be adjacent to dense scar tissue in the host.

3) Band-shaped, or other types of opacity in eyes affected by active uveitis.

4) Dystrophia adiposa The implant is invariably invaded by the dystrophy and consequently becomes opaque.

5) Deep extensive *tear-gas burns especially if* the eye is irritable and photophobic and if blepharospasm and lacrimation are present

6) Deep extensive tattoo-like opacities caused by explosions

7) Opacities in aphakic eyes in which opacities are too deep for lamellar grafting, especially if intracapsular extraction has been performed A full-thickness graft in such an eye is frequently complicated by incarceration of the iris lens capsule or vitreum with subsequent development of cloudiness or opacity If incarcerations do not develop some of these grafts may preserve transparency

8) Extensive opacities with superficial pannus-like vascularization usually caused by chemical, *flame, or molten-metal burns* and accompanied by variable degrees of symblepharon and often intensive photophobia. Transplants in these eyes become opaque.

9) Advanced Fuchs epithelial dystrophy extensive opacities with calcareous degeneration and opacity due to pemphigus.

10) Opacity accompanied by anterior synechias if they are combined with increased intraocular pressure If tensions can be normalized by preliminary operation such eyes offer improved prognosis.

11) Opacity of many years duration, especially if it has been present since shortly after birth These eyes usually have pronounced nystagmus so that even though transplants may remain clear a high degree of amblyopia prevents visual improvement.

### Preliminary Treatment of Unfavorable Eyes

When the condition of an eye is very unfavorable to keratoplasty and the graft would otherwise almost certainly remain cloudy a preliminary operation may improve the cornea structurally and make it more favorable for keratoplasty for visual improvement. If a cornea is superficially opaque and vascularized the outer layers including the greater part of the abnormality can be excised, using a partial or total keratectomy The open blood vessels at the periphery of the keratectomy are then treated by beta irradiation to prevent revascularization These procedures may improve the condition of the cornea sufficiently so that when the eye has become quiet a keratoplasty can be carried out with a fair promise of success (figure 72)

If an eye is severely scarred by burns and is *affected not only by dense vascularized leukoma* but also by symblepharon, the latter should be treated first by plastic repair with or without




FIG 72 Cases unfavorable for keratoplasty rendered more favorable by preliminary surgery A Extensive vascularized corneal opacity B Partial lamellar keratectomy C After partial penetrating square keratoplasty

D Dense vascularized corneal opacity and symblepharon E After total lamellar keratectomy and beta irradiation F After partial penetrating circular keratoplasty

mucous membrane transplants. The vascularized opacity should then be treated by lamellar keratectomy Beta irradiation should be applied after operation to prevent the recurrence of vascularity Finally the cornea, thus greatly improved is in a more favorable condition for an optical keratoplasty (figure 73)

If the symblepharons are not large, the bulbar conjunctiva, tarsus, and fornix can be repaired by sliding conjunctival flaps from the adjacent area. However conjunctival material may not be sufficient for repairing both bulbar and tarsal regions if the symblepharon is very large. In such cases, the shortage is made up by free mucosal grafts taken from the same individual's buccal mucosa material from the lower lip or lateral mouth surface is the best substitute for conjunctiva. The buccal graft should be cut slightly larger than the defects to be covered and should be very thin if either of these conditions is not met, structural correction and cosmetic effect will not be optimal. After it has been cut from the mouth the graft can be placed on the finger with the epithelium next to the finger The graft is then thinned as much as possible with fine scissors until little remains but minimal supportive tissue and the epithelium itself A graft thinned in this manner is easily handled It is placed in the eye trimmed if necessary to fit the defect, and sutured in place. In suturing it is important to anchor each stitch to the sclera to insure smooth cicatrization and to prevent contraction of the graft.




FIG 73 Very unfavorable case for keratoplasty made more favorable by preliminary surgery A Very dense vascularized corneal opacity with extensive symblepharon B After plastic repair of conjunctiva with buccal mucous membrane graft C After total lamellar keratectomy and beta irradiation D After partial penetrating circular keratoplasty

After a few months the graft resembles normal conjunctiva, or at worst, slightly congested conjunctiva. If however it has not been sufficiently thinned, it tends to contract and remains red thick and unsightly

Some eyes in the unfavorable group cannot be improved with a view to keratoplasty because of complicating conditions due to intraocular disease which cannot be corrected. Densely opaque corneas with extensive or total anterior synechias are of this type. However if such eyes are not affected by secondary glaucoma and if light per

ception and projection remain good, a total penetrating keratoplasty offers some chance of restoring vision and is the only procedure which does offer any promise (figure 74)

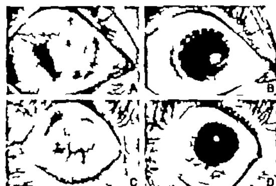



Fig 74 Cases very unfavorable for keratoplasty with extensive vascularized corneal opacities and extensive anterior synechia A and C Before B and D After total penetrating keratoplasty

**Retransplants**

The graft may become cloudy because of protrusion poor nutrition during recuperation, anterior synechias, or postoperative uveitis. If complications which might interfere with permanent transparency of a new graft, such as opacity or vascularization, have not appeared in the surrounding cornea, these eyes may be improved by a second or sometimes by more retransplants (figure 75) A second transplant should not be considered, however until the eye has become quiet after the first operation. At least six months, preferably a year should be allowed.

If the first operation failed because the technique was erroneously selected, the failure may be corrected by using a more suitable technique for the second operation. For example, if the first graft performed to correct keratoconus was not large enough to remove all, or almost all, of the conus the graft may have remained transparent but because it was set in a protruding




Fig 75 A. Opaque partial penetrating square keratoplasty B Opaque partial penetrating circular keratoplasty within previous graft C Third successful partial penetrating circular keratoplasty




Fig 76 A Case of advanced keratoconus B and C After partial penetrating square keratoplasty The graft was too small to remove the whole conus resulting in poor vision D and E After a larger partial penetrating square retransplant which restored normal corneal curvature

area, it may have resulted in myopia or astigmatism severe enough to counteract any visual improvement due to the clarity of the graft. A second transplant larger than the first should improve the cornea structurally and restore the curvature to normal or near normal and therefore insure better vision (figure 76)

## KERATOPLASTY FOR PURPOSES OTHER THAN VISUAL IMPROVEMENT

### Therapeutic Keratoplasty

If an irritable, inflammatory or degenerative corneal disease does not respond well to conservative treatment, keratoplasty may stop or shorten the evolution of an acute torpid state or a recurrent lesion. Among the conditions which may benefit from therapeutic keratoplasty are corneal abscess, herpetic and disciform keratitis acute interstitial keratitis and traumatic lesions. If traumatic lesions are perforated or if descemetocele or perforation are imminent in cases of progressive ulceration emergency transplantations are indicated (figure 77)

### Reconstructive Keratoplasty

A reconstructive graft is indicated when the eye is very unfavorable to keratoplasty because the cornea is in poor condition. The reconstructive graft is used to improve the cornea structurally and is followed by a final optical keratoplasty Severe burns are the best indication for this type of graft. Superficial layers of opaque vascularized cornea are excised using partial or total lamellar keratectomy and are replaced by a lamellar graft of the same dimensions as the excised corneal tissue. If the lamellar graft does not improve vision enough for practical purposes the structural improvement of the cornea renders the eye more favorable for a final partial lamellar or penetrating keratoplasty performed to improve vision

### Keratoplasty for Cosmetic Improvement

Occasionally eyes with corneal opacity dating from early infancy and therefore very amblyopic

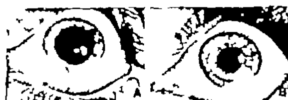



Fig 77 Case of descemetocele A Before B After partial penetrating circular keratoplasty

may require a partial corneal transplantation only to improve the eye cosmetically A total penetrating keratoplasty may be performed in some blind eyes with extensive staphyloma in a final attempt to improve appearances before resorting to enucleation.

### Trophic Keratoplasty

Corneal transplantation may have a beneficial effect on the host tissues and in some instances may clear areas of opacity adjacent to the graft. For this reason some surgeons have implanted corneal tissue next to grafts which have become cloudy in order to clarify them, or in the hope of speeding the clearing process which would naturally occur in time if no irreversible changes, such as fibrosis have developed

## CONCLUSION

Clinical keratoplasty in man is no longer in an experimental stage. Thousands of cases reported have proved that corneal grafts can be successful in a very high percentage of favorable cases when suitable techniques are used. The indications for keratoplasty are becoming more numerous almost daily Many unfavorable cases which only a few years ago were dismissed as unsuitable for successful grafting can at present be rehabilitated by combined procedures of plastic and conjunctival repair keratectomy and keratoplasty

The possibility of directly observing clear and opaque corneal grafts and of correlating these observations with other experimental data and with microscopic examination of experimental and clinical material offers a marvelous opportunity for investigation in the field of transplantation. From such data investigators in other fields may gain valuable information

# PART IV

# *Fat*

CHAPTER 6

# *Transplantation of Fat*

LYNDON A PEER

## I ADIPOSE TISSUE

Judging by the scarce and controversial literature and the small amount of space devoted to adipose tissue in histology text books, the study of fat cells and fatty tissues has been a neglected subject. Perhaps histologists and biochemists have exhausted the old experimental methods, and other avenues of approach are required to provide a better understanding of the origin, behavior and metabolic functions of fat cells.

The fate of fat cells following transplantation as free grafts described in this chapter may be one of these new approaches to clarification of certain important aspects of fat cell behavior

### Occurrence of Fat in Plant and Animal Cells

Fat is a normal constituent of all animal tissue cells. For example the Golgi element and mitochondria in the cytoplasm of cells both contain fatty materials, hence they are generally dissolved in the course of preparing a microscopic section and tend to appear as clear spaces (1) Fat droplets are also present in the cytoplasm of cells and these droplets are likewise dissolved when fat solvents are used in the preparation of histologic sections. With osmic acid and other stains numerous fatty particles can be demonstrated in the cytoplasm of phagocytic cells which accumulate in areas where freely transplanted human fat grafts have broken down (2)

Intracellular fat in larger amounts occurs in fat depot regions such as the intramuscular and abdominal subcutaneous tissues Storage depots are absent, however in the intracranial membranes and eyelids where an appreciable increase in fat-cell content might cause disastrous intracranial pressure or interfere with vision.

In the vegetable kingdom fats occur in seeds and fruits and sometimes in roots.

### ORIGIN AND DEVELOPMENT OF ADIPOSE TISSUE

#### Different Types of Adipose Tissue

According to Fawcett (3) *white adipose tissue* begins to develop subcutaneously in certain areas, called *fat islands*, of particularly vascular mesenchymal cells, during the fourth and fifth months of embryonic life. Many small lipid droplets appear in the cytoplasm of cells which seem to be primitive fibroblasts. As the lipid droplets accumulate in the cytoplasm, the primitive fibroblast cell withdraws its process and rounds out. The droplets enlarge and coalesce to form a large single drop which displaces the nucleus to one side and the cytoplasm to the periphery Sections of such cells have the form of signet rings.

*Multilocular or brown adipose tissue* is a second type of fat, which is distinguishable from ordinary adipose tissue by its light brown color and its lobular gland-like appearance This so-called *brown fat* is most extensively developed in hibernating animal species and thus is sometimes called the *hibernating gland* (4) but fatty tissue having the same gross structure and microscopic




FIG 78 Leukocytes and phagocytes found in inflamed tissues 1 neutrophil granulocytes 2 eosinophil granulocytes 3 lymphocytes 4 plasma cells 5 macrophages filled with engulfed fatty material— foam cells 6 foreign body giant cells with included fatty droplets and crystals ×750 (From R A Willis The Principles of Pathology p 52 C V Mosby Company St Louis, 1950 )



FIG 79 Fat cells differentiating in the fifth month from the connective tissue near a capillary ×250 (From L B Arey Developmental Anatomy ed 4 p 362 W B Saunders Company Philadelphia 1941 )

appearance is found in a number of non-hibernating mammals and may occur in man (5)

Sidman (6) examined the normal embryologic development of brown adipose tissue in 18-day old fetuses and in older animal Differentiation of the interscapular brown fat was similar *in vivo* and *in vitro* Brown adipose tissue differentiated *in vitro* in cultures of mesenchyme from rat fetuses in which this type of fatty tissue cannot yet be recognized by morphologic *criteria*. When cultured in synthetic media immature brown fat differentiates and retains an appearance typical of brown fat for over two weeks. When grown in serum the cells progressively accumulate cytoplasmic lipid and after a week may resemble ordinary white fat cells. *Cells which migrate from the explants look at first like fibroblasts but then accumulate fat more rapidly and may acquire the appearance of white fat cells*

The experiments were interpreted as evidence that brown adipose tissue 1) can differentiate in the absence of nervous or endocrine influences 2) *is determined while still part of the loose mesenchyme* and in this respect *is as specific a tissue as muscle cartilage and bone* and 3) does not *differ fundamentally from white adipose tissue.*

The glycogen and lipid contents of the cells of brown adipose tissue vary markedly with the *physiological state of the animal. In further* experimental work by Sidman (1956) fragments of the interscapular brown fat body from rat *fetuses* and newborn rats were *maintained in* organ culture and insulin was added. Insulin has a direct effect on organ cultures of brown adipose tissue of rats. After several days *in vitro* in natural or synthetic media, the tissue possesses the enzymatic apparatus for effecting glycogen synthesis. In all media insulin prolongs survival of the tissue *in vitro* as well as increases glycogen synthesis and lipid deposition (7)

During embryonic life brown adipose tissue develops in definite parts of the body as well defined masses of tissue with a rich blood supply When such cells take on fat it is first deposited in fine granules, such as in fatty degeneration or as in the lipid-rich cells of the adrenal cortex. As the granules become larger the cell takes on a moruloid or "mulberry" appearance Only with the most extreme deposition of fat do all the droplets fuse into one or a few large fat spaces and often the nucleus retains its central location and does not take on the flattened form at the cell periphery characteristic of the ordinary adipose tissue. In rats, in mice and in hibernating animals the brown adipose tissue cells commonly exhibit the mulberry appearance throughout life irrespective *of how fat the animal becomes.* In man brown fat may be present in the dorsocervical and interscapular regions. Hibernoma a *tumor of the so-called hibernating fat gland* or organ in human beings, consists of multilocular or mulberry cells with separate deposits of fat *in the cytoplasm* of the cells and a centrally located nucleus (8)

## Theories on the Origin of Fat

Fully formed fat cells apparently do not have the power to divide, and this may be regarded as evidence of their high degree of specialization. New adipose tissue is said to develop in postnatal life, as it does in the fetus, from spindle-shaped cells in loose connective tissue (3) There is still no general agreement, however as to the identity of the cells from which it arises. Some believe that they are undifferentiated mesenchymal cells which persist along the walls of small blood vessels (9) others regard them as reticular cells, and a few believe they are *specific lipoblasts* set apart early in embryonic life for fat storage or fat cell replacement.

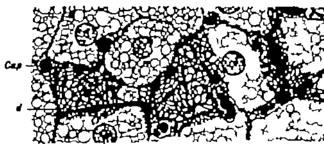



FIG 80 Brown fat tissue from the hibernating gland of a white rat *Cap* Capillary *d* intercellular fibrous network Impregnation method of Hortega ×900 After Nageotte and Guyon Note that nucleus is centrally placed and fat is in multiple fine droplets instead of single large globules (*From A A Maximow and W Bloom A Textbook* of Histology ed 3 p 68 W B Saunders Company Philadelphia, 1940 )

Mm. longissimus dorsi et ilio-costalis lumborum
M quadratus lumborum
M. latissimus dorsi
M psoas
Fascia lumbalis
Fascia transversalis
M obliquus externus abdominis
M obliquus internus abdominis
M transversus abdominis
Vertebra lumbalis IV
Peritoneum
Fascia transversalis
Aponeurosis mm. obliqui interni et transversi abdominis
Linea alba
B
Peritoneum
Fascia transversalis
Linea semilunaris
Anterior layer of rectus sheath
Posterior layer of rectus sheath
Vagina (sheath) m. recti abdominis
M rectus abdominis
Linea alba
A

FIG 81 A Above the linea semicircularis (of Douglas) the anterior and posterior rectus sheaths are equally formed from the aponeuroses of the lateral muscles B Below the linea semicircularis there is no posterior rectus sheath but the anterior rectus sheath is formed and supported by all the aponeuroses of the three flat lateral muscles and the subcutaneous fatty layer The adipose cells in this layer serve as *storage receptacles for lipid from ingested food Old storage fat* is removed and oxidized and new dietary fat is stored in cells which have given up their lipid

In obesity the cells take on fat and *increase greatly in size but probably do not increase in numbers* In starvation the adipose cells give up lipid and decrease to such an extent that they resemble plump fibroblasts with centrally located nuclei and a few fat droplets in the cytoplasm (From L C Callander Surgical Anatomy ed 2 p 278 W B Saunders Company Philadelphia 1939 )

## SPECIFICITY OF FATTY TISSUE

Fat is not laid down in all connective tissues where fibroblasts are present but it particularly selects certain regions—such as the subcutaneous tissues the omentum, and the mesenteries of the peritoneum (10) This selective deposition of fat may perhaps indicate that it accumulates in specific cells which have a definite regional distribution rather than in ordinary fibroblasts which form a constituent of connective tissue everywhere. Such a conclusion is corroborated by experimental observations.

For example if a piece of *apparently indifferent connective tissue* normally destined to form a local deposit of fat is transplanted to some other part of the body where similar accumulations of fat do not occur *it still becomes differentiated into adipose tissue* (11) The specificity of fat cells is further suggested by the occasional occurrence of fat tumors (lipomas) which develop as circumscribed benign growths irrespective of the development of adipose tissue in the body generally

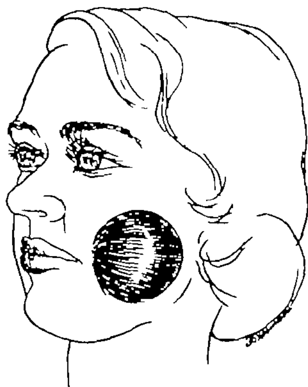


Fig 82. Lipoma in cheek region These benign fatty tumors are said to retain their fat during starvation when adipose cells in fat depots elsewhere are giving up fat

Another example of regional differences in the behavior of fatty tissues is the fact that the administration of female hormones causes fat cells in specific locations to take on more fatty bulk and thus produce regional feminine curves.

Malignant fatty tumors offer an undoubted instance of accumulation of fat despite the most severe depletion of fat from the normal fat depots (5) Contrariwise, it is interesting to note that in certain tumors fibroblasts which do not differentiate into adipose tissue early in development do not later exhibit any ability to accumulate fat and develop into fatty tissue.

The sucking pads in emaciated infants may become extremely prominent because they are not absorbed as is the subcutaneous fat. What enables the fat to remain here when the fat elsewhere is being depleted is an unsolved problem Whether lipomas resist absorption of their fat content in emaciated individuals is still a matter of debate.

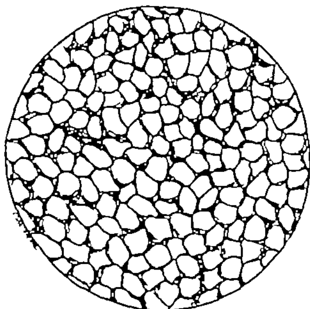


Fig 83 Drawing illustrating normal appearance of fat cells in a lipoma

Wells (5) in his fascinating article Adipose Tissue A Neglected Subject, states that fat has been the subject of relatively little study The most fundamental problem, the origin and nature of adipose tissue, is still a matter for some disagreement. From time to time for many years anatomists, pathologists and embryologists have been discovering and rediscovering the fact that fatty tissue is not merely a common connective tissue loaded with simple stored fats but is to a large extent structurally developmentally and functionally an independent tissue more on the order of the ductless glands

The early belief, emphasized by Virchow in his book *Cellular Pathology* contended that adipose tissue is merely ordinary connective tissue in which fat has been deposited. This seems to be the view still generally held by those who have given no particular consideration to the subject, and it involves the assumption that any and all connective tissue can and does serve as a repository for fat when this accumulates in excess.

A little reflection, however shows *the impossibility of this conception*, because fat does not become generally and diffusely deposited in the connective tissues throughout the body even in obesity For example, imagine the complications that might occur if the eyelids of obese individuals took on the same amount of subcutaneous fat as the abdominal wall or if the cerebral membranes became as thick as the omentum. The hands, the feet, the ears, and the nose are seen to undergo much less thickening in obesity than the abdominal wall thighs, buttocks, and shoulders.

## Clinical Evidence of Fat Specificity

An interesting demonstration of the individuality of fatty tissue was reported by Strandberg (12) in 1915 and this has been verified by a number of later investigators.

A girl aged 12 years lost the skin from the dorsum of one hand through a burn The defect was covered by a pedicled graft from the abdominal wall which included both skin and subcutaneous tissue containing fat The result was satisfactory for a time but as the girl matured and acquired the rotundity of a matron the transplanted subcutaneous tissue failed to realize its altered status and took on the same amount of fat as the abdominal wall

This phenomenon has been of very common occurrence in the author's experience when flaps of abdominal skin and subcutaneous fat were transferred to the hand neck or other regions of the body It is a real problem in pre-adolescent girls with transplanted abdominal flaps who take on abdominal fat due to evening and between meal snacks milk shakes and the like Transplanted fat on the *dorsal* or palmar surfaces of the hand in such patients assumes a 'boxing glove' appearance and transplanted abdominal fat to the neck region forms unsightly rolls

Apparently the transplanted abdominal fat follows the same physiologic behavior as the fat cells in the abdominal wall depot which represented the donor site from which the pedicled flap was transplanted If the patient takes on an increase in abdominal fat the transplanted fat also participates in this increase. Conversely, if the abdominal fat is reduced the fat content in the transplanted fat cells is also reduced and this indicates that it is good judgment not to remove any large portions of the transplanted fat When the pre adolescent girl becomes 'boy conscious' she will restrict her diet and reduce the fat content both in her abdominal fat cells and in the transplanted abdominal fat

*In the author's experience free transplants of human abdominal adipose tissue also take on fat in their new location when the abdominal donor site takes on fat*

One fat graft transplanted within the rectus sheath actually increased in weight and volume although free fat grafts in general lose about 50 per cent of their weight and volume in eight to twelve months following transplantation due to the failure of some fat cells to survive the transfer In this case the patient took on an increase in abdominal fat after operation and the free fat graft in the rectus sheath participated in this increase (2) In all probability the graft contained fewer fat cells but those which survived took on a larger amount of fat

Wells (5) is prepared to accept the view that even while still distended with fat the fat cell may be carrying on important functions not as yet disclosed because the mere presence of a load of fat need not seriously impair other activities. In such distended cells there are presumably just as much nuclear material and cytoplasm as there were before the fat was deposited, and these functioning elements, being outside the fat, *are in immediate contact with the blood supply over a much greater area than in the fat free cell*

The gland-like characteristics of brown fat, the fact that fat grafts from the abdominal wall transplanted elsewhere take on fat when the abdominal wall tissues take on fat, and many other observations previously mentioned, strongly suggest the specialized structure of adipose tissue and its probable origin from mesenchymal cells that in some degree are specific cells, embryologically predestined to become fat cells.

One must bear in mind that whereas the regular and generally accepted behavior of the germ layers is apparently a valid concept in its broad outline numerous exceptions do exist. In other words cells have a rather fluid potentiality during early development The actual

product, whether fat or some other tissue depends on the environment in which the germ cells find themselves as well as on their origin (1)

Those who support the specificity theory of adipose tissue predicate that the fat cell is a definite and specific type of cell formed by differentiation from mesenchymal cells that are destined to take on fat and become specialized fat cells. This school of thought holds that *the fat cell is not a fibroblast does not arise from one and in emaciation does not revert to a fibroblast*

## HISTOLOGY OF WHITE ADIPOSE TISSUE

Most histologists are in essential agreement on the classification and anatomic structure of adipose tissue. Matters concerning the embryogenesis, development, life cycle and replacement of the fat cell, however are still subjects of debate.

Adipose tissue is characteristically organized as well defined lobes and lobules,* each being encapsulated in a delicate sheath of collagenous fibers and surrounded by a network of capillaries. In the embryo this vascular network is already well established in regions *where fat will later accumulate even before the fat is deposited* (10)

### Cells and Intercellular Substances

Angevine (13) observes that all of the soft connective tissues including fat are composed of a homogeneous *matrix* or ground substance in which are incorporated certain fibers and cells and it is only the relative preponderance of either one of these elements (fibers or cells) that determines the classification of the tissue. If one distends connective tissue with fluid or air it immediately becomes evident that there are numerous spaces between the various fibers and it is in this area that the ground substance resides

In addition to the ground substance there is *tissue fluid* from the blood plasma, which contains water-soluble proteins, metabolites crystaloids, gases, and so forth. This water borne traffic must pass through all anatomic barriers from capillary to cell and back again through the walls of lymphatics or venules if the cells are to function properly and even to survive. Just how this two-way exchange of substances necessary for cell life is accomplished remains a mystery

The character of soft connective tissue† varies greatly in different parts of the body depending upon the relative proportions and arrangements of its cellular fibrous and amorphous components. Thus tendon and deep fascia are pliable and strong because of the preponderance of tough bundles of collagenous fibers between the sparsely located tendon and fascia cells. If one were to mask the collagenous fibers in tendon and fascia by introducing calcium salts or some sort of homogeneous mucoprotein, the tissues would resemble the hard connective tissues, bone or cartilage (with some difference in parenchymal cell structure and arrangement) In cartilage however *all blood vessels*, lymphatics, nerves and wandering cells which are present in tendon and fascia would have to be removed, since cartilage contains only one living unit, its parenchymal chondrocyte.

In contrast, the specialized connective tissue fat, *has a preponderance of cellular elements*, and the characteristic white or yellow color is due to the intracellular fat in its fat cells which *are the parenchymal cells of adipose tissue.* Adipose tissue, with its preponderance of cellular elements arranged in lobules rather closely *resembles the specialized connective tissue* skeletal muscle, which is likewise largely made up of cellular constituents similarly divided into bundles or lobules. Both the individual muscle cells and the individual fat cells are supported by a connective tissue stroma which has its own fibroblast cell, and it is this stroma which contains blood vessels and nerves supplying the individual fat and muscle cells.

### The Fat Cell

A typical fat cell appears as little more than a thin envelope of cytoplasm surrounding a relatively large globule of fat

According to Fawcett (14) because of the unusually large size of fat cell a histologic

* Every surgeon knows that grossly the subcutaneous adipose tissue in infants and young children appears more finely granular (has smaller lobules) than the adipose tissue in older children and in adult Free skin grafts applied over subcutaneous adipose tissue are more often complete takes in infants and young children than when *similar skin grafts are applied in adults*

† Connective tissue proper which does not include hard connective tissue such as bone and cartilage

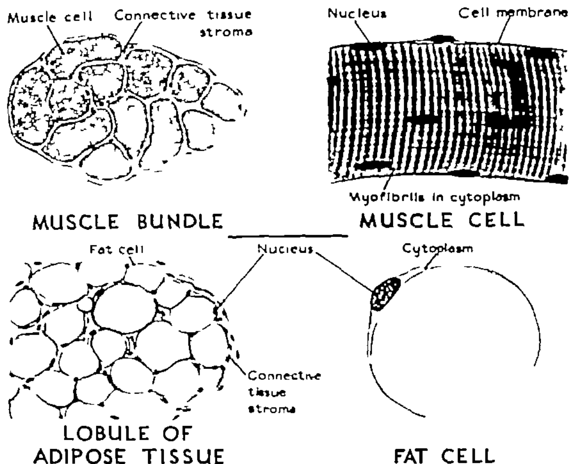



FIG 84 Individual white adipose cells like skeletal muscle cells are divided into lobules or bundles by supporting connective tissue which contains the vessels and nerves This connective tissue also surrounds each individual fat and muscle cell

The fat cell has a signet ring appearance owing to the fact that the nucleus is displaced with its cytoplasm to the cell periphery by the large fat globule the globule is storage fat and it decreases in size and is again replenished in regular cycles of about 11 days (Clark and Clark)

section of usual thickness includes only a thin segment of those fat cells that are transected, and the nucleus of only a few cells that happen to be within the plane of the section. The majority of the cells therefore may appear to lack a nucleus. Histologic sections may also be misleading in respect to the shape of the fat cells because extraction of lipid during preparation of adipose tissue for paraffin imbedding frequently leads to collapse of their delicate membranes and a consequent distortion of their shape. Isolated fat cells in fresh areolar tissue are spherical but when they are crowded together in adipose tissue they mutually tend to deform one another into polygonal shape.

With special staining methods, rod-shaped and filamentous *mitochondria* can be demonstrated in the thicker portions of the cytoplasm adjacent to the nucleus and in the thinner portions around the lipid vacuole (14) The lipid which consists of a mixture of glycerides of fatty acids, *remains fluid at body temperature* but may solidify on cooling, with the formation of sheaves of thin needle-like crystals.

Although it is difficult to believe that the tenuous layer of *protoplasm* in the fat cell is the site of complex chemical reactions, biochemical and histochemical observations suggest this probability (15) In addition to droplets of neutral fat stainable by sudan black, the cytoplasm of fat cells contains phospholipid demonstrable by Baker's acid hematein stain. The cytoplasm also has a positive histochemical reaction for enzymes, alkaline phosphatase esterone, and succinic dehydrogenase. In animals fed after a period of fasting a transient accumulation of glycogen is found in the cytoplasm of fat cells, and there is reason to believe that this glycogen is subsequently reduced to small molecular units which are used by the fat cells in the synthesis of lipid (15)

During a prolonged period of fasting fat cells




FIG 85 A Human buccal fat Note that individual fat cells and groups of fat cells are surrounded by supporting connective tissue stroma which contains the vessels and nerves Large artery and vein are seen in upper part of section ×100 B Higher magnification of buccal fat cells showing occa ional nuclei at cell periphery ×400

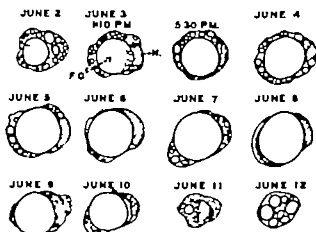



FIG 86 Camera lucida records of a single new fat cell (Clark and Clark courtesy of Am J Anat ) (From L V Cowdry A Textbook of Histology ed 4 p 41 Lea & Febiger Philadelphia 1950 )

in such storage depot as the subcutaneous tissues the mesenteries and the omentum give up their lipid to supply the metabolic requirement of other tissues If fasting continues, the fat droplets may entirely disappear with reversion of the fat cell to a cell which some authors consider indistinguishable from a fibroblast but which others consider different from a typical fibroblast.

## Life Span of Fat Cells

Histologists sometimes state rather generally that tissue cells are constantly dying and being replaced While this statement is accurate regarding blood cells and epidermal cells in the skin it apparently does not apply to cartilage cells and may not apply to fat cell Cartilage cells, after they stop dividing, probably remain viable until the individual s death Death and replacement are not known to occur among the cells of peripheral nerve and skeletal muscle and it is possible that human fat cells likewise have a similar long life expectancy unless destroyed by injury or disease

## Blood Vessels and Nerves

Fat is generally regarded as a poorly vascularized tissue but one mu t understand that the

metabolic activity of a fat cell is confined to its narrow rim of cytoplasm. Although this cytoplasm probably extends completely around the external circumference of the cell, it constitutes only a small portion of the total cell volume, which is largely occupied by the fat globule.

The total capillary bed in adipose tissue is only about one-third as great as in muscle tissue. *But if the ratio of capillary bed to volume of cytoplasm is calculated, one finds that the amount of blood supply in proportion to the volume of cytoplasm is actually greater in adipose tissue than in muscle tissue* (16)

Surgeons often speak of the subcutaneous abdominal tissue as being relatively avascular and go on to say that for this reason the abdominal site is not a favorable recipient area for free grafts In the author's experience the abdominal adipose tissue is not particularly avascular from a gross standpoint it often bleeds rather profusely in moderately lean patients Most of the author's experimental grafts of septal bone cartilage tendon nerve fascia and other tissues were buried in abdominal fat and in most instances they healed cleanly and survived as well as when buried elsewhere (in rectus muscle neck fat and so forth) Free surface skin grafts however do not take well over abdominal fat because broken down fatty material and fluid form beneath the graft If an opening is made in the skin graft and the fluid is removed the graft may receive blood vessel anastomosis from vessels in the fat and "take" even five days after transplantation Thus skin cells can apparently remain viable at body temperature without any circulating blood up to five days after transplantation provided they are in a moist environment

Undoubtedly free fat from injured fat cells is an irritant when present as an intercellular substance This indicates the clinical importance of gentle handling of adipose tissues in all surgical manipulations

Nerves, blood vessels and probably small lymphatic vessels are present in the narrow spaces between the closely packed fat cells. The vessels and nerves are supported by a delicate connective tissue stroma In Fawcett's opinion (17) this stroma consists of fine reticular fibers.* The larger connective tissue spaces between lobules are occupied by collagenous, elastic and reticular fibers with scattered fibroblast cells which represent the parenchymatous cells of this connective tissue stroma The subcutaneous adipose tissue in the soles of the feet and palms of the hand contains a larger number of elastic fibers than other adipose areas for obvious functional purposes.

* Reticular fibers are now accepted as young or immature collagenous fibers and both types are believed to be produced through direct activity of the fibroblast (18) The exact origin of elastic fibers is not known and apparently after early development new elastic fibers are not formed

Mast cells eosinophils and occasional lymphocytes have been observed in the interstitial spaces between fat cells. In transplanted fat grafts a tremendous increase occurs in the number and variety of this intercellular population as will be described later under human fat grafts.

## CHEMICAL CONSTITUENTS OF FAT

Fats that occur in animal and vegetable cells are chemically identified as glycerol esters of various fatty acids Fatty acids combine with glycerine to form fats and oils.

The fixed fats and oils are a well defined group of substances which are characterized by all gradations of physical consistency ranging from oils that are fluid even below the freezing point of water to hard fats which melt at 50°C

Hence, no sharp distinction can be made between fatty oils† and fats. Nevertheless it is convenient to apply the term "oil" to those glycerides which are fluid below 20°C and the word "fat" to those which are solid above this temperature. As a general inclusive term the word "fat" as employed here is preferable, since this avoids confusion with mineral and essential oils (10)

† Lewkowitsch and Warburton (19) observe in their interesting chapter that the fatty oils always of paramount importance as food acquired an increased significance during World War II as sources of glycerine one of the basic chemicals in explosives of the dynamite class The shortage of edible oils in all countries during the war led to great advances in the technique of oil refining Oils hitherto regarded purely for industrial use became available for food as a result of improved methods of purification It may be said today that almost all fats with the exception of those markedly active physiologically e g castor curcas and chaulmoogra oils can be utilized as food In Germany attempts were made to supplement available resources with fat obtained from yeast under intensive cultivation Synthetic fatty acids can be prepared by the oxidation of paraffin wax and other hydrocarbons

The "waxes" are esters formed by the combination of one molecule of fatty acid with one molecule of a monohydric alcohol such as cetyl alcohol, cholesterol, etc They have characteristic physical properties both visual and tactile which have led to the creation of the term "waxy appearance or waxy feel."

### Factors Affecting the Chemistry of Stored Fat

The oils and fats should not be regarded as fixed and definite chemical constituents that always remain the same in a given species or kind of plant or animal (19) Their chemical composition varies within narrow limits, according to climate and soil in the case of plants and in the case of animals according to the climate, the race, the age of the animal, and especially *the kind of fat in the animal's food.* The milk fat of cows fed on a diet rich in coconut oil resembles the latter oil in its properties, and the fat of the Eskimo has a high iodine value and simulates blubber It is probable however that fat which is synthesized in the body from carbohydrates and proteins is chemically constant for a given animal.

### Synthesis and Mobilization of Fat

In a study of mice and rats Favarger (20) ascertained that the role of the liver in the total synthesis of fat is more important in a state of alimentary equilibrium than during restoration of the reserves. The liver synthesizes fatty acids with a certain retardation because no precursor is elaborated elsewhere. On the basis of his observations Favarger considers that in a general way the role which the liver plays in the synthesis of fat has been much overestimated. From the entire evidence available he concludes that the elaboration of fats is accomplished mainly *in the fatty tissue itself*

As viewed by Tracht, Goldstein and Ramey (21) under the stimulus of fasting, depot fat is mobilized for dissimilation in the liver and peripheral tissues. Using differences in the epididymal fat body of the rat as a measure of depot fat changes these investigators attempted to evaluate the role of both the liver and adrenal steroids in the movement of fat out of peripheral depots. The results of their investigation indicate that fat mobilization is not inhibited by adrenalectomy In the absence of the liver however movement of fat from depots is altered. It is suggested that the liver in some way regulates the movement of fat from storage areas in response to changes in the caloric needs of the animal.

## PHYSIOLOGIC PROCESSES

### Absorption of Fat in Animals and Man

The processes underlying the absorption of fat have long been a subject for debate, and many aspects of the problem still remain obscure (22) It now appears to be established that except for small quantities which may be absorbed as a fine emulsion of unsplit fat, hydrolysis of fat into its constituent fatty acids and glycerine is a necessary step prior to its absorption. After absorption across the epithelial boundary the fatty acid combines with the glycerine and appears in the intestinal epithelial cell as sharply outlined droplets which give characteristic staining reactions (23) An increasing accumulation of fat droplets at first small and then larger develops above the nucleus of the cell during absorption. Thus the large proportion of fatty acids and glycerine in the intestinal lumen are absorbed through the striated border of the cell and are at once synthesized into neutral fat. Normal intestinal absorption of fat is impeded by vitamin B deficiency

The synthesized fat finds its way by an unknown process into the lacteals (lymphatics) of the villi and through the lymphatics into the blood circulation *via* the thoracic duct. Only a small part (about 20 per cent) of absorbed fat, however can be collected from the thoracic duct lymph (22) Several investigators have reported that some fat is absorbed directly into the portal system but this is still a very controversial point. Satisfactory data which can account for all the fat that disappears from the intestine have not yet been presented, but evidence has been introduced by the Mayo Clinic group which shows that in the rat practically all of the absorbed fat may be detected in the lymph pathway

Fat in the lacteals is called chyle and the term chylomicrons is used for those tiny droplets which can be seen in blood plasma after a fatty meal when light is reflected from their surfaces in dark field microscopic examination The term chylomicrons is appropriate because the droplets enter the blood stream in the lymph or

chyle, having come from the area of intestinal absorption (24) Chylomicrons have been thoroughly studied by Gage* and Fish (25) and their article which is a valuable contribution to the subject, should be read by all who are interested in adipose tissue Evidence has been provided by Ludlum, Taft and Nugent (26) that the fat is encased in a protective film of protein which may condition its utilization whether for storage or oxidation.

### Deposition of Animal Body Fat

Until recent years it was commonly thought that reserve fatty tissue was laid down only during periods when the animal ingested food in excess of its caloric requirement According to this conception the constituent glycerides of depot fat remained as they had been formed originally unless they were called upon to meet the energy demand of the animal. As viewed by Longenecker (27) however the experimental findings of Schoenheimer and his collaborators have made this idea untenable, and in its place the concept of a depot fat which is constantly in a state of flux has arisen.

Schoenheimer (28) alone, and Schoenheimer in collaboration with Rittenberg (29) utilized the fact that fatty acids can be marked by inclusion of heavy hydrogen (deuterium) In experiments with mice, kept on a carbohydrate diet plus heavy hydrogen, stored fats in the cells were replaced in about six days by new fatty acids containing heavy hydrogen. These fatty acids may be either exactly the same structurally as the displaced components or entirely different. In other experiments it was found that a large part of dietary fat *is not oxidized directly but is first deposited in the fat cells replacing old fat, which is removed and oxidized.* Thus a continuous source of fatty acids is available for this dynamic metabolism of the fatty tissue either from food fat or by conversion of carbohydrates or proteins into fat through a process of synthesis. It is probable that fat synthesized *de novo* from carbohydrates and proteins is constant for a given animal.

Cowdry (30) notes that fat cells do not exist merely as containers with energy-rich material locked up in them at some remote period like the coal strata in a mine Both microscopic and chemical evidence show that fat in storage is systematically changed. When single cells were held under observation in special chambers inserted in the ears of rabbits by the Clarks (31) they were seen to undergo cyclic changes involving accumulation of fat in small droplets coalescence of these to form a single large droplet, subsequent decrease in size of the droplet followed by repetition of the same process again and again. These cycles may be relatively short in some instances and perhaps longer in others. Cowdry states that the assumption of some investigators that fat cells are short lived is unwarranted *Dividing fat cells are scarcely ever seen The same may be said of dying ones*

Boecke (32) has demonstrated sympathetic nerve fibers running to individual fat cells which can be shown to be distinct from the nerve supply for blood vessels in fatty tissue and which may even penetrate the fat cell. Beznak and Hasch (33) observed that section of the nerve supply to a fat area modifies the behavior of the fat cells in storing and yielding fat, and it is therefore the sympathetic fibers that control the deposition and mobilization of fat in adipose tissue.

It is difficult, however to evaluate the importance of the sympathetic nerve control over the deposition or removal of fat in free and pedicled abdominal transplants which are grafted in such different locations as the hand and neck, or as free grafts within the rectus sheath The blood supply seems to be of more importance, since free abdominal grafts with a completely interrupted blood supply undergo considerable initial breakdown, whereas abdominal pedicled flaps with attached blood supply retain most of their fat content after they have received a new blood supply from the recipient site and the pedicle has been severed *Both the free abdominal fat graft and the transplanted abdominal pedicled graft tend to take on fat if the abdominal fat is increased*

Normally the storage and release of fat are regulated to a nicety in each region in amounts established by custom. If more fat is absorbed than is oxidized it is heaped up If oxidation is increased over absorption fat is decreased. When the rate of metabolism is geared up by administration of thyroxine, stored fat is burned. As viewed by Julian and his associates (34) anterior pituitary hormone causes migration of body fat to the liver while lipocaic hormone of the

* The author as a first year medical student served as one of Professor Gage's guinea pigs in his study of chylomicrons (1922)

pancreas facilitates movement of fat to body depots. Other hormones may also be important as in activation of the mammary gland after pregnancy.

The work of Mason, Dam, and Granados (35) suggests that vitamin E, possibly acting as an antioxidant, enables fat cells to incorporate and stabilize fatty acids as storage fat.

## FUNCTION AND DISTRIBUTION OF ADIPOSE TISSUE

### Function of Fatty Tissue

In recent years investigators have clarified many aspects of fatty synthesis and fat storage in adipose tissue. Increasing attention has been directed to the possibility that adipose tissue is an actively functioning organ and part of a system subject to its own diseases. Adipose tissue, in addition to its known function as a storage depot, may also be a manufacturing plant in active operation, not only producing some or all of its own stored materials but possibly conducting other biochemical processes not yet revealed.

The most evident function of fats in animal organisms is to supply immediate food requirements or to provide food reserves which supply by subsequent oxidation, energy for growing and working tissues. The storage of fats and oils in vegetable seeds may be similarly explained as a food reserve for the plant embryo.

It has been stated that the evidence collected during the past twenty years has stressed the metabolic potential of adipose tissue (36). The oxygen consumption of this tissue is inversely proportional to the fat content and varies directly with the nitrogen content of the depot. The level of metabolic activity is determined by dietary, neural and endocrine factors. The metabolic service of adipose tissue to the body economy would appear to be twofold. Adipose tissue accumulates the triglycerides from the diet and liver that are carried by the blood stream to the depots and stored there until mobilized by as yet unknown mechanisms. The other more important function to the body may be the conversion of blood glucose to fatty acids that are stored as triglycerides for future use. Recent research indicates that the latter function may be of great importance to an understanding of the biochemical basis for obesity.

Adipose tissue is an important site of lipogenesis. When the differences between the lipid and the protein content of adipose tissue and liver were taken into account the former proved to be far more active in glucose oxidation and lipogenesis.

That liver and adipose tissue should be stimulated to convert glucose to fatty acids by the same dietary factors would indicate that the same humoral or hormonal factors are operating at these widely separated sites (36).

Not only does adipose tissue provide fat storage for reserve energy-yielding material but it also serves as an insulator against rapid heat loss in cold weather, as well as a light weight packing material for other tissues and organs.

The fats and especially the waxes render other valuable service by virtue of their physical properties. For example, beeswax prevents dilution of the concentrated sugar solutions of the comb by external moisture; waxes and, in some instances, fats serve as thin coverings for the leaves and fruit of plants, thus preserving the underlying tissues from loss of water.

In vegetable seeds and fruits the formation of fats occurs late in the ripening process. In the unripe condition carbohydrates (sugar, starches, etc.) but no fatty acids (or oils) are present in the fruits and sap. It is probable that these carbohydrates are broken up and converted into fatty acids and subsequently into glycerides during the maturing process. In almonds it has been observed that carbohydrates disappear as fat is being formed.

### Distribution of Adipose Tissue

In a well nourished individual fat is not laid down in all connective tissues where fat is present, but, as previously mentioned, particularly selects certain regions such as the subcutaneous tissues, the omentum, the breasts and the mesenteries of the peritoneum.

Adipose tissue tends to be absent in the cerebral membranes, eyelids, and scrotum. Adipose tissue in the hands, feet, ears and nose is seen to take on much less fat in obesity than the fat on the abdominal wall, thighs, buttock and shoulders.

It is also to be noted that when fat is deposited in the adult it tends to be laid down first in the neighborhood of blood vessels. Moreover, the study of fat formation in living tissues has shown that it develops primarily in regions where the circulation is moderate or sluggish. On the other hand, it tends to diminish rapidly when the

circulation in adjacent blood vessels *becomes overactive* an observation that affords some evidence for the effectiveness of massage exercise, and the local application of heat for decreasing subcutaneous adipose tissue (10)

Fatty tissues vary in respect to firmness according to their supportive function. Kuhns (37) observed that abdominal fat cells are surrounded by a loose connective tissue stroma which serves more as a packing material than as supporting tissue for the fat cells. In special areas such as the heel, finger tip thenar eminence, and patella, the fatty connective tissue serves to support the fat cells. In these locations it contains more elastic fibers than ordinary adipose tissue so that the fat resumes its shape after pressure is removed. This elastic adipose tissue is well adapted to withstand sudden impacts and prolonged pressure, such as occurs when an individual works with tools stands on his heels or kneels for long periods of time. Observations have shown that this fat is spared during nutritional demands made upon other fatty depots in the body and, conversely that it does not tend to take on large increases in obesity

*In replacing the skin and fat in these special areas one substitutes not only a different type of skin but also a different type of fat which is not constituted to withstand sudden pressures and weight bearing Unfortunately additional elastic fibers are not formed in the transplanted fat, and it must be protected against excessive and sudden pressures. The transplanted skin also retains the qualities of its donor site and does not assume the character of palmar or plantar skin. This indicates that both fat and skin are endowed with specific regional characteristics which are tenaciously retained when they are transplanted to other areas of the body*

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In experiments on rats Rehn (14) in 1912, implanted homogenous fat grafts of cherry size taken from the inguinal region under the dorsal fascia Partial or *total suppuration of the fat grafts occurred in one-third of the animals.* In some the homogenous transplant healed without reaction Observations were made at intervals over a period of 8 to 24 weeks. After 8 weeks there was distinct shrinkage of the transplant. The processes almost exactly paralleled the changes seen in inflammatory and proliferating atrophy of fatty tissue. Rehn noted the disappearance of the fat from the fat cells and multinuclear fat cells in sections without indication of any cell infiltration from the stroma Numerous young cell formations were seen as well as vascular connective tissue septa separating individual fat lobules, the newly formed tissue being rich in cells. The central section showed complete necrosis and the peripheral parts, cystic degeneration The first signs of beginning regeneration were noticeable and after 12 weeks the young forms of future fat cells were clearly seen.

In 1912 Makkas (15) from the Surgical Clinic of Bonn used, as a rule, the lower end of the femur in dogs as the site of implantation of autogenous fatty tissue. A pad of fat taken from the subcutaneous tissue of the hypogastrium of the same animal was pressed in place as a tampon. (No homogenous implants were made.) The results were positive in 7 of the 8 experiments, the fat healing in and the osseous cavity being entirely filled necrosis occurred in one case. The duration of observation of these 7 experiments lasted 20 35 51 56 60, 68 and 91 days respectively The results were successful even when the pieces of implanted fat were the size of a hen's egg

In many animals the fat was replaced by connective tissue shortly after implantation in others the fatty tissue was maintained for a long time For the most part the filled-in connective tissue was firm and poor in cells. Sometimes it appeared myxomatous and became vascularized In all preparations except one compact osseous substance was found between the fat implant and the surrounding spongiosa

In experiments on dogs Sumita (16) in 1912, laid wide somewhat thick pedicled fat flaps from the external side of the thigh in the hip joint after removal of joint cartilage Other tissues such as fascia alone pedicled fat containing fascia tendon, tendon sheath and muscle were also used. The experiments showed that the placement of soft parts in joints resulted in bony stiffening but functional effects were *always obtained. All pedicled flaps of soft parts taken from the near neighborhood maintained their viability* The interlaid flaps of soft parts showed a uniform fibrous change whether fascia tendon tissue fat or muscle. *The fat tissue lost its normal appearance in a short time and was gradually replaced by connective tissue*

Rehn (17) (1913) made an important comparative study of fat homoplastics in rabbits and autoplastics in dogs. *The investigations showed no essential differences,* those differences evident were more quantitative than qualitative properties. Wide tissue septa with vessels and nerve trunks invaded the autogenous fat implants and also the homogenous grafts. In homotransplants of fat, healing sometimes occurred without reaction and at other times small regions developed abscesses. *The autotransplants of fatty tissue had a strong tendency to heal in without reaction.* A large part of the transplant retained all its components unchanged it was retained as it was at the outset and became a permanent part in its recipient. In sections after 30 days the transplanted fatty tissue was observed to have undergone changes familiar in inflammation and atrophy of fatty tissue.

Hilse (18) of the Faculty Clinic in Dorpat began his experimental work with free transplantations of fatty tissue in rabbits in June 1913 the results being confirmed in dogs. Nineteen autogenous fat transplants for the most part taken from the groin and freed of fascia were spread on severely bleeding defects in the liver spleen and kidney so that the edge of the graft extended beyond the large wound surface After one to three minutes bleeding stopped. No further bleeding was observed even in animals sacrificed in 2 to 5 days. Sometimes a hematoma formed There was prompt firm adhesion of the fat transplant through formation of blood coagula and the graft healed in without reaction. Control of the bleeding could be achieved by adhesion of the fat transplant alone without suture. Hilse believed that these results could not be explained by tamponic effect of the transplanted fat alone, although this cannot be entirely excluded. *He suggested a specific blood coagulating effect.*

Previous clinical application of free fat graft to joints affected by ankylosis and arthritis

deformans was experimentally confirmed by Röpke (19) in 1913 Into osseous cavities in joints from which sections had been removed he successfully implanted autogenous fat transplants taken from the groin. Perfect healing occurred in all animals. Reunion of the joint ends was prevented.

Everywhere in the newly created joint the fat graft heals in and is soon surrounded by a dark connective tissue capsule, from which the fasciculi of connective tissue, forming a mass, advance into the transplant and more or less extend to take in the region of the implanted fat graft. Where the fat can be maintained functionally fat tissue is also seen after waning of the regenerative processes. About the twenty fourth week the regenerative process in the transplant is terminated, so that at this time fatty tissue is found in fat islands histologically similar to normal.

In a preliminary publication Lawrowa (20) (1913) reported on experiments with rabbits in which transplants of fatty tissue and muscle had been carried out. In tamponized cavities thus filled, development of bone and osseous marrow was found on the twenty fourth day

In experiments on rabbits by Polenow (Poljeloff) and Ladygin (Lodygin) (21) in 1913 fatty tissue was found to have hemostatic properties against severe bleeding of the kidneys liver spleen and lungs. Subcutaneous or peritoneal fatty tissue was sutured to the margins of the wound after loss of substance without further tamponade. The hemorrhages stopped almost on contact with, and under the influence of transplanted fatty tissue.

In his research on skin bone, muscle, fatty tissue, and all other tissues and organs Donati (22) (1913) observed that the preservation of tissue fragments of the rabbit outside the body in Ringer's solution or in agar serum, at freezing temperature from 2 to 5 days did not cause the appearance of autolysis. Furthermore, the behavior of the grafts remained substantially the same histologically whether the tissue fragments were inserted immediately or after they had been preserved for 2 to 5 days. The myelin figures were seen only in a few bits of subcutaneous fat after they had remained in agar serum for 48 hours, but they had no effect on the behavior of these pieces when they were implanted.

Experimenting with rabbits Eden and Rehn (23) (1914) severed nerves and tendons in different regions and covered the area with autogenous subcutaneous fatty tissue taken from the inguinal region. After varying periods of time the nerve and tendon were again exposed, and the suture area was excised with its adjoining tissue for examination. Observations extended from 16 to 369 days. In all transplants the fatty tissue had the same appearance and mass as the original transplants The transplanted tissue was closed off from the surrounding tissue by a capsule. After 369 days the fatty tissue was completely normal only here and there the fat septa and the connective tissue layer separating the peritoneum from the transplant were somewhat widened

Broeckaert and Steinhaus (24) (1914) of the University of Gand transplanted 4-day-old cultures of small fragments of living fat in autogenous plasma under the dorsal aponeurosis of the rabbit. The results were negative in almost every instance. Two cultures remained active for a long time. The first graft disappeared rapidly the second did better After a survival of 40 days no trace of the graft was found Conclusively these experiments demonstrate that fat grafts, removed from the same or other subjects of the same species in general take very well During the first days the graft increases in size, then retracts and disappears after a certain number of weeks. The larger the mass implanted, the longer the time required to be absorbed, *homogenous grafts of fat being absorbed more rapidly than autografts*. Nevertheless Broeckaert and Steinhaus noted that certain grafts, perhaps being more resistant than others when placed under favorable conditions, continued to be viable for a longer period of time. They believed that a fat graft is useful even though it plays only a temporary role.

In their histopathologic study of autogenous and homogenous fat grafts at varying periods of 17 to 91 days, Broeckaert and Steinhaus found that the duration of the grafts in the tissues did not regularly parallel structural changes. Three phenomena were observed namely connective tissue proliferation around and in the graft, slight infiltration chiefly by lymphocytes and serous atrophy of the fat cells.

Ohkohchi (25) (1914) carried out experiments on the control of bleeding from the kidney with transplanted autogenous fatty tissue from the vicinity The fat itself remained viable while at the wound surface there was a small strip of

completely necrotic parenchyma. In another instance of hemorrhage from the liver after a week the fat graft adhered firmly to the wound but the center of the transplant was necrotic After 12 days an intermediate layer of connective tissue was present on the wound surface. Proliferation extended into the fatty tissue and was active in neighboring parenchyma After 17 days the wound began to shrink, and the fatty tissue changed to connective tissue.

Autogenous and homogenous fat grafts from the abdominal wall were used to cover liver wounds In four days the transplants adhered weakly to the wound round cell infiltration blood pigment, and islands of necrosis at the edge of the liver wound were observed. On the ninth day the intermediate connective tissue layer was well developed. After the twelfth day the healing had progressed so well that both autogenous and homogenous grafts were penetrated by connective tissue and vessels and the infiltration had disappeared

The free transplantation of autogenous fat grafts from the groin into the knee joint in rabbits was investigated by Eisleb (26) (1916) In the resected knee joint the transplanted fatty tissue showed many changes. It was converted into more or less differentiated connective tissue the structure of which was favorable for function in the joint. Parts of fatty tissue which had been maintained were as they had been when originally transplanted other areas of the fat grafts disintegrated and later were again built up through a process of new-fat formation.

In experiments on rabbits, Henze and Mayer (27) (1916) laid a piece of fat taken from the inguinal region of the operated animal around the ends of the tendons and interwoven silk. The leg was put at rest, and after 10 11 13 and 60 days sections were examined. In all 4 animals adhesions were present, even after 10 days corresponding to the fibrous metaplasia of fat. Similar experiments were carried out by inserting different tissues and substances.

In an investigation by Lawrowa (28) in 1917 the medullary cavity produced in rabbits by removal of the marrow was filled with autogenous fatty tissue omentum or muscle. After periods of one day to 200 days bone sections were examined On the fourth day the connective tissue cells were active in destroying the content of the fat cells The fatty tissue in its earlier form was lost Within some months there was further development of connective tissue elements in the transplant associated with detritus and young fat cells had begun to develop. Lawrowa concluded that in the transplantation of fatty tissue in osseous cavities the graft was destroyed after which connective tissue and later bone marrow occur in its place The osseous defect is replaced by newly formed osseous tissue which stems from periosteum, the process lasting for some months Age and individuality play a large role.

In experiments on dogs Koll (29) (1917) sutured or tied autogenous or homogenous fat into or over wounds produced in the kidney by crushing and laceration or over nephrotomized and decapsulated kidney Some of the transplants became infected with colon bacilli and others with staphylococci. Bleeding stopped promptly The kidneys were removed at periods varying from one day to six months. Fat transplants in pockets made in the bladder wall were examined. In each instance the fat rapidly disintegrated and disappeared from these formed pockets. Sections from the areas where fat had been transplanted showed a typical metaplasia of reticular connective tissue becoming fibrous tissue. Grossly in most instances of nephrotomies secondary contraction reduced the kidney from one-half to one-quarter of its normal size In 24 hours the fibrin deposits showed beginning organization. The change into fibrous connective tissue was complete in 3 to 4 months. Infection did not alter the metaplasia though it delayed it.

After citing Rehn's observations (made on Lexer's inducement) of homoplastic and autoplastic fatty tissue in rabbits and dogs Lexer (30) in his comprehensive exposition on fat transplantations in 1919 summarized the histologic changes. A part of the autogenous fat transplant is maintained unchanged whereas homogenous fat transplants undergo generalized changes. After cell atrophy with proliferation of nuclei, cellular infiltration and cystic degeneration the young forms of future fat cells prevail. These young forms are derived partly from old fat cells and partly from the connective tissue transplanted with them and initiate regeneration slowly While after a period of 100 days an autotransplant again appears a living fatty tissue *homotransplanted fat at this point shows complete retardation of reparative and regenerative manifestations* The first 10 to 12 weeks one can designate as the degeneration period localized

in autografts and widespread in homografts. *The regeneration period begins in both forms after 7 to 8 weeks* The variety of serum and the cell albumin of different individuals are considered to have an important role in the process.

Working with dogs Kloso (31) (1922) of the University of Frankfurt a. M sutured fat implants to the edges of the pericardium. The earliest transplant was examined 15 days after a period of smooth, normal healing. Microscopic sections from the border zone confirmed that the entire part of the fatty tissue turned toward the heart in places had been converted into an inner connective tissue layer rich in cells which advanced into the fatty tissue. Thirty-six-day old sections from the middle of the fat implant showed large islands of connective tissue from the border zone of the implant. Large vessels with profuse vascular buddings leading to the pericardial stump were evident.

Neuhof (32) (1923) after an extensive review of the literature on fat transplantation, stated that transplanted autogenous fat undergoes practically the same changes as transplanted bone. The fat transplant dies and is replaced either by fibrous tissue or by newly formed fat arising from host cells, which take on fat and become fat cells. The evidence is in favor of the regenerated fat being of metaplastic origin despite the fact that most investigators maintain that a small part of the transplant survives and that regeneration is derived from this source.

After interposing fat in the knee of rabbits, Magnus (33) (1924) noted that the tissue is quickly destroyed and replaced by connective tissue. The secondary joint is differentiated by the effect of function on the connective tissue. Magnus pointed out that the tests on animals are of limited value since the resected joints were not stiffened Histologically the reconstructive process appeared in full course after four and a half years. He considered that the period of time was too short to indicate late end-results.

Experimenting with rabbits, Bertocchi (34) (1923) used as implants homogenous adipose tissue from the axilla, groin, and omentum or kidney capsule, and some that had been previously fixed in formalin and preserved in alcohol. The grafts were placed in the outer skin of the rabbit's ear next to the central auricular artery Specimens from the implants were examined from 5 to 120 days after fixation On the tenth day slight diminution was noted in the graft, and this shrinking continued at a fair rate until the thirtieth day when it became slower At the end of two months the size of the graft was reduced to nearly half the original size. At the end of four months it was very difficult to find any difference between the site of the graft and the surrounding region The implanted tissue became enclosed in a capsule of cicatricial tissue and was gradually replaced by newly formed connective tissue throughout, but this occurred much more slowly in the preserved implants than in fresh implants. As stated by Bertocchi, even in regions previously free from fat new fatty tissue formed from connective tissue.

Rossi (35) (1925) carried out 20 experiments on dogs transplanting autogenous muscle and fatty tissue in pulmonary wounds. The animals were sacrificed at varying periods of 2 to 90 days, and sections were examined. At the end of 3 days the fat transplant did not adhere to the wound but the deep part was the site of an active proliferation of lung connective tissue. In 9 days adhesions were present in some locations, which microscopically corresponded to mild infiltration of fat by new connective tissue. *In 15 days the transplanted adipose tissue was absorbed* On the fortieth day the wound was closed by a compact connective cicatrix which presented only traces of the transplanted fat. The lung tissue proper showed no extraordinary reactions except new formation of its connective tissue. The fat transplant was well tolerated by the lung. Rossi preferred muscle transplant to fat transplant.

Histologic studies on free fat transplants in rabbits were made by Hilse (36) (1928) of Riga who concluded that in all free transplantation of fatty tissue *regeneration of adipose tissue occurs through the activity of infiltrating histocytes from the host cells*

Histocytes predominate over polymorphonuclear and round cells after three days. Formation of giant cells occurs after 10 days with very few polymorphonuclear leukocytes. Histocytes, endothelial cells and giant cells are present in greater number than in the earlier period. After 13 days there is granulation tissue after 19 days the connective tissue fibers are smaller and the nuclei more spindle shaped. Similar changes are observed after 20 days.

In further experiments on rabbits by Bertocchi (37) (1929) preserved autogenous and homogenous fat peeled from the kidney axilla, and

inguinal fossa and omentum were implanted in the marrow of the femoral diaphysis. Healing occurred by *first intention. Homogenous as well* as autogenous implants were well tolerated. The animals were sacrificed and sections examined at varying periods of 5 to 150 days. In 5 days the implant of homogenous fat was united with the host tissue. In 10 days the fat had become striated. The graft was reduced in size and was subdivided by a network from the surrounding compact capsule. There was marked densification of the periosteum. In 60 days the endosteum reacted causing foci of medullary calcification and thickening of the cortex.

Even at the stage of 50 days the fibroblasts held small drops of fat which merged into one large drop. The nucleus was confined to the periphery and a typical drop of fat formed secondarily. At the end of 80 days, and to a greater degree at the end of 120 to 150 days the normal marrow fat was restored.

Preserved autogenous and homogenous fat grafts were implanted by Ceri (38) (1932) in the thoracic and abdominal wall of dogs. *There was no substantial difference between autoplastic and homoplastic tissue when previously preserved.* Following inflammatory reaction the graft gradually diminished in size without cicatricial retraction. Microscopically the graft showed a lymphocytic stage with elimination of dead elements followed by a fibroblastic stage. Fibrous *connective tissue formed and became well* vascularized, dividing the fat graft into small sections. Small islands of fat cells remain scattered throughout the newly formed tissue. *It was impossible to distinguish whether these fatty cell islands represented fat changes from invading histiocytic elements of the host or were deposits of fat in the pre-existing cells of the graft.*

Davis and Traut (39) maintained that the histologic changes which occur in transplants of fat closely approximate those which occur in transplanted bone. These authors stated that *none of the transplanted fat actually survives* but after a primary degeneration it is entirely replaced by an embryonic type of cells which later develops into fat cells or by fibrous connective tissue.

A somewhat neglected but extremely valuable experimental study concerning the behavior of free autogenous and homogenous fat transplants in white rats was reported by Charles E. Gurney (40) in 1937. Autotransplants of fat from the groin which were taken without trauma, multiple small pieces, small traumatized pieces and single untraumatized homogenous pieces of fat were transferred to the ventral thoracic region and examined at varying periods of one to three weeks and of one to twelve months. Single pieces of autogenous fat taken from the peritoneal cavity were examined at intervals of 1 week and 1, 4, 9 and 12 months. *Autografts which were not traumatized retained their identity as fat throughout the year of observation.* At the end of a year transplants of fat from the groin had become about one-fourth their original size, those of testicular fat, about one-half. Autografts cut into multiple small pieces and those subjected to trauma had begun to disappear at the end of 2 months and, with one exception, were entirely gone at the end of 6 months. *The homografts disappeared entirely by the third month.*

Microscopically certain portions of the surviving grafts were becoming vascularized as early as one week after transplantation. Other portions of the graft degenerated and disappeared leaving only apparently normal fat. The histocyte seemed to be the chief phagocytic cell of the fat which was liberated by the degenerating fat cells. No giant cells were found in any section. No new formation of fat cells was seen. Thus Gurney concluded that *some of the fat cells in autogenous grafts survive transplantation and these constitute the fatty tissue that eventually remains.*

*In a series of experiments* Hausberger (41) (1938) transferred 43 testicular and 22 ovarian fat bodies of three- to four-day-old rats to the inner side of the abdominal wall of other rats, male and female. At this stage (three days after birth) the testicular and ovarian fat bodies still have the same structure and the cells are morphologically still undifferentiated. Each host received two homotransplants from the donor. The grafts were examined in 1 to 73 days after transplantation. A typical testicular fat body developed from the cell material of the testicular fat body and a typical ovarian fat body developed from the cell material of the ovarian fat body. Whether transference was from a male to female or *vice versa* made no difference.

In the same way transplanted connective tissue does not develop into fatty tissue. On the basis of transplantation results it is accepted that the measure of fatty tissue growth, its quantity and form are conditioned partly by *the factors*

anchored in the tissue itself The size of the fatty depot partly depends on those mechanisms which as endogenous and exogenous influences affect the depots That is to say the further typical development of young testicular and ovarian fat bodies can be conditioned only by factors which rest within themselves. *The fat cells show differentiation from connective tissue cells and are specific cells*

According to Hausberger (42) (1939) if the free fat and testicular bodies and ovarian fat compounds of young rats are transplanted to the extraperitoneal abdominal wall of older animals of the same species, then accumulations of lipoma-like fat from the germinal tissue or growth formations of a connective-tissue-like nature can develop The final size reached 500 mg i.e one-fourth of the body weight of the host animal. Growth was not observed beyond the fourth month. A germinal layer transferred under the scalp reached only a weight of 50 mg. four weeks after transplantation.

Livermore (43) of the University of Tennessee in 1939 transplanted autogenous muscle and fat into the kidney of the dog. He concluded that foreign material introduced into the kidney parenchyma causes infarction deposition of calcium, and damage to the kidney tissue. *Both muscle and fat undergo degeneration and replacement by connective tissue* The muscle transplants cause more damage than fat.

Clark and Clark (44) (1940) studied the histologic details of fat formation and its increase and decrease in the living rabbit by round table" chambers, which were kept under observation for at least four months after installation. Fat frequently appeared first in close proximity to blood vessels. After new fat had formed it showed a tendency to diminish during or immediately following a period of active circulation and to increase again with the return of quiet circulation. No relationship was seen between the formation, increase, or decrease of fat and the presence or absence of lymphatic capillaries. New fat developed from fibroblast-like cells present in the area for weeks or months. *Whether these cells were specific, potential fat cells or whether they were the ordinary connective cells could not be determined.* Mitotic division of fibroblasts in the intervascular tissue was repeatedly observed.

The pre-adipose cell was an elongated cell with processes which acquired a number of minute refractile droplets and then withdrew its processes and became rounded. Droplets increasing in number coalesced to form larger globules and later a single large globule with the nucleus crowded to one side in the same cell was seen The loss of fat showed a reverse process—reduction in the size of the globule, its break-up into smaller and smaller droplets and disappearance of the latter leaving a large cell containing granules. *Later this same cell again took on fat and became a typical fat cell* No change of a former fat containing cell into a fibroblast-like form was observed Fat apparently entered the cells in a soluble form and not by phagocytosis of visible fat globules.

In further experimental work by Hausberger (45) (1941) the testicular fat bodies of four to six-day-old rats were buried subcutaneously in the same animal and in other animals of the same species. The subcutaneous homotransplantation of these morphologically undifferentiated testicular adipose deposits (and other young fat depots) formed mostly connective tissue which grew many hundredfold in size. *Pure adult fatty tissue seldom arose* Autotransplantations on the other hand, *always developed into lipoma-like nodules arising from pure fatty tissue* The growth in size was greater in the autotransplantations. *Homotransplantations* began to regress at the latest 4 months after transmission while autotransplantations still showed no involution after 14 months.

Continuing his experimental studies of adipose tissue Hausberger (46) in 1941 reported that there was no change in weight in the fat depot of adult rabbits, rats, and mice 14 days to 8 months after removal of the testicle, ovary or kidney on one side. If the operation was carried out on young growing rats, the fatty tissue on the side without the organ was sometimes decreased.

Hausberger pointed out that the capacity of fatty tissue to build up fat from carbohydrates has been demonstrated (1941) Conclusively, a series of factors directly affecting the fatty tissue can influence the assimilation of fat. It is very conceivable that such mechanisms are inherited and thus represent the direct causes of fat deposition

Menschik (47) (1944) divided mice into groups of controls, vitamin-E-deficient animals and

inguinal fossa and omentum were implanted in the marrow of the femoral diaphysis. Healing occurred by first intention. Homogenous as well as autogenous implants were well tolerated. The animals were sacrificed and sections examined at varying periods of 5 to 150 days. In 5 days the implant of homogenous fat was united with the host tissue. In 10 days the fat had become striated. The graft was reduced in size and was subdivided by a network from the surrounding compact capsule. There was marked densification of the periosteum. In 60 days the endosteum reacted, causing foci of medullary calcification and thickening of the cortex.

Even at the stage of 50 days the fibroblasts held small drops of fat which merged into one large drop. The nucleus was confined to the periphery and a typical drop of fat formed secondarily. At the end of 80 days and to a greater degree at the end of 120 to 150 days the normal marrow fat was restored.

Preserved autogenous and homogenous fat grafts were implanted by Cieri (38) (1932) in the thoracic and abdominal wall of dogs. *There was no substantial difference between autoplastic and homoplastic tissue when previously preserved.* Following inflammatory reaction the graft gradually diminished in size without cicatricial retraction. Microscopically the graft showed a lymphocytic stage, with elimination of dead elements followed by a fibroblastic stage. Fibrous connective tissue formed and became well vascularized, dividing the fat graft into small sections. Small islands of fat cells remain scattered throughout the newly formed tissue. *It was impossible to distinguish whether these fatty cell islands represented fat changes from invading histocytic elements of the host or were deposits of fat in the pre-existing cells of the graft.*

Davis and Traut (39) maintained that the histologic changes which occur in transplants of fat closely approximate those which occur in transplanted bone. These authors stated that *none of the transplanted fat actually survives* but after a primary degeneration it is entirely replaced by an embryonic type of cells which later develops into fat cells or by fibrous connective tissue.

A somewhat neglected but extremely valuable experimental study concerning the behavior of free autogenous and homogenous fat transplants in white rats was reported by Charles E. Gurney (40) in 1937. Autotransplants of fat from the groin which were taken without trauma, multiple small pieces, small traumatized pieces and single untraumatized homogenous pieces of fat were transferred to the ventral thoracic region and examined at varying periods of one to three weeks and of one to twelve months. Single pieces of autogenous fat taken from the peritoneal cavity were examined at intervals of 1 week, and 1, 4, 9 and 12 months. *Autografts which were not traumatized retained their identity as fat throughout the year of observation.* At the end of a year transplants of fat from the groin had become about one-fourth their original size, *those of testicular fat about one-half. Autografts* cut into multiple small pieces and those subjected to trauma had begun to disappear at the end of 2 months and, with one exception, were entirely gone at the end of 6 months. *The homografts disappeared entirely by the third month.*

Microscopically certain portions of the surviving grafts were becoming vascularized as early as one week after transplantation. Other portions of the graft degenerated and disappeared leaving only apparently normal fat. The histocyte seemed to be the chief phagocytic cell of the fat which was liberated by the degenerating fat cells. No giant cells were found in any section. No new formation of fat cells was seen. Thus Gurney concluded that *some of the fat cells in autogenous grafts survive transplantation and these constitute the fatty tissue that eventually remains.*

In a series of experiments Hausberger (41) (1938) transferred 43 testicular and 22 ovarian fat bodies of three- to four-day-old rats to the inner side of the abdominal wall of other rats, male and female. At this stage (three days after birth) the testicular and ovarian fat bodies still have the same structure and the cells are morphologically still undifferentiated. Each host received two homotransplants from the donor. The grafts were examined in 1 to 75 days after transplantation. A typical testicular fat body developed from the cell material of the testicular fat body and a typical ovarian body developed from the cell mat[illegible] ovarian fat body. Whether tran[illegible] a male to female or *vice versa* [illegible]

In the same way tran[illegible] tissue does not develop into [illegible] basis of transplantation result[illegible] the measure of fatty ti[illegible] and form are conditioned [illegible]

anchored in the tissue itself The size of the fatty depot partly depends on those mechanisms which as endogenous and exogenous influences affect the depots. That is to say, the further typical development of young testicular and ovarian fat bodies can be conditioned only by factors which rest within themselves. *The fat cells show differentiation from connective tissue cells and are specific cells*

According to Hausberger (42) (1939) if the free fat and testicular bodies and ovarian fat compounds of young rats are transplanted to the extraperitoneal abdominal wall of older animals of the same species, then accumulations of lipoma-like fat from the germinal tissue or growth formations of a connective-tissue-like nature can develop The final size reached 500 mg i.e one-fourth of the body weight of the host animal. Growth was not observed beyond the fourth month. A germinal layer transferred under the scalp reached only a weight of 50 mg four weeks after transplantation.

Livermore (43) of the University of Tennessee, in 1939 transplanted autogenous muscle and fat into the kidney of the dog He concluded that foreign material introduced into the kidney parenchyma causes infarction, deposition of calcium, and damage to the kidney tissue. *Both muscle and fat undergo degeneration and replacement by connective tissue* The muscle transplants cause more damage than fat.

Clark and Clark (44) (1940) studied the histologic details of fat formation and its increase and decrease in the living rabbit by round table" chambers, which were kept under observation for at least four months after installation Fat frequently appeared first in close proximity to blood vessels. After new fat had formed it showed a tendency to diminish during or immediately following a period of active circulation and to increase again with the return of quiet circulation. No relationship was seen between the formation, increase or decrease of fat and the presence or absence of lymphatic capillaries. New fat developed from fibroblast-like cells present in the area for weeks or months. *Whether these cells were specific potential fat cells or whether they were the ordinary connective cells could not be determined* Mitotic division of fibroblasts in the intervascular tissue was repeatedly observed.

The pre-adipose cell was an elongated cell with processes which acquired a number of minute refractile droplets and then withdrew its processes and became rounded. Droplets increasing in number coalesced to form larger globules and later a single large globule with the nucleus crowded to one side in the same cell was seen The loss of fat showed a reverse process—reduction in the size of the globule, its break-up into smaller and smaller droplets and disappearance of the latter leaving a large cell containing granules. *Later this same cell again took on fat and became a typical fat cell* No change of a former fat containing cell into a fibroblast-like form was observed Fat apparently entered the cells in a soluble form and not by phagocytosis of visible fat globules.

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Hausberger pointed out that the capacity of fatty tissue to build up fat from carbohydrates has been demonstrated (1941) Conclusively a series of factors directly affecting the fatty tissue can influence the assimilation of fat. It is very conceivable that such mechanisms are inherited and thus represent the direct causes of fat deposition.

Menschik (47) (1944) divided mice into groups of controls vitamin-E-deficient animals, and

vitamin-E-rich animals. The animals fed on vitamin-E-deficient fat-rich diet gained weight very slightly and developed some adipose tissue only during the first 40 to 70 weeks of the diet. Thereafter each vitamin-E-deficient animal started to lose weight between the thirty-seventh and seventy-fifth week of the experimental feeding. After 1 to 114 weeks at least one of each diet group was sacrificed. Autopsies in this vitamin E-deficient group after 64 days on an average showed that the subcutaneous and subperitoneal adipose tissue had disappeared. Female mice fed on the same fat-rich diet supplemented by *vitamin E (α-tocopherol) after 45 to 65* weeks gained considerable weight. Fatty connective tissue was observed in many regions as under the skin of the dorsum of the body, on the lateral sides and around the thorax.

In experiments with the bilateral folds of omentum-like tissue attached to the testes of rats, Cameron and Seneviratne (48) (1947) of the University College Hospital Medical School, London, tried to determine whether adult fatty tissue regenerates under the stimulus of partial removal of the parent mass.

The abdominal cavity was opened and about one-half of one or two testicular omenta were removed. The animals made uninterrupted recovery. Studies of histologic changes in the stump were made at varying times after resection. The conclusion was that *mature fat cells possess no proliferative capacity* although new fatty tissue can be laid down by modification of cells in close association with blood vessels. *These investigations give no support to the idea of regeneration of adipose tissue since neither grafts nor traumatized omental white fat showed proliferation of fat cells although repair by fibrous tissue production took place in both.*

Cameron and Seneviratne think that the view of adipose tissue as a purely inert depot with little metabolism of its own ought to be abandoned.

*Smelser and Ozanics (49) injected anterior* pituitary extracts into nine thyroidectomized guinea pigs producing exophthalmos and edematous orbital fat tissues. Homogenous orbital fat transplanted into the orbit behaved in the same manner as native orbital fat and to the same degree. Fat unaffected in its normal location (axilla) by injection of anterior pituitary retains this characteristic when transplanted to the orbit of the same animal.

The fats of animals have been shown to originate in part by synthesis from dietary carbohydrate and protein, and in addition, the dietary fat is generally considered to exert a marked effect in their composition (Shoreland).

Shoreland (50) says that contrary to current conceptions, certain animal fats, such as beef and mutton tallow, are not appreciably modified by the nature of dietary fat. He divides animal fat into two types: 1) Fats the fatty acid composition of which is substantially unaffected by the nature of the dietary fat, as in beef and mutton tallow. Designation of such fats by the term "heterolipoid" to indicate lack of resemblance to the dietary fat has been proposed. 2) Fats which readily incorporate the fatty acids present in the dietary fat. For such fats the term "homolipoid" is suggested to designate similarity in composition to that of the ingested fat. Most animal fats apparently belong to the latter type but some are intermediate in type in that while their composition is not very readily altered by the nature of the dietary fat, nevertheless by feeding certain oils such as rapeseed oil it is possible to incorporate small proportion of fatty acids that are not normally found in such fats.

Fromme (51) (1951) of Dresden points out that the development of every organism does not end with the completion of growth but that cell *replacement (regeneration)* must take place during the entire life as substitute for the cells degenerating to their death. The potency of cells to divide decreases with the years. It is false, however, to assume that in the old no more young cells capable of regeneration are present, and that an old body is completely matured. Cells capable of regeneration are maintained in definite places up to the greatest age.

*The success of a free transplantation depends on the presence of tissue capable of regeneration and therefore of surviving.* This capacity to regenerate depends on the presence of mesenchymal tissue which is especially sensitive to external influences. Therefore the greatest amount of preservation of a transplant and parent tissue is necessary. Fromme further feels that *the time of day and meteorologic conditions are also factors to be considered.*

Gohrbandt (52) (1952) of Berlin considers that the success of a homotransplantation is measured not only by its healing on or healing in for a *definite time. The transplant must become an*

integral element of the new host, participating in his biologic functions rather than merely taking a parasitic role.

Gohrbandt emphasizes that dead tissue cannot come to life again ("dead is dead" as he puts it). If however conditions are made favorable, dead tissue can heal in its new host and take over certain functions which have no relation to the life of the tissues. Dead homoplastic tissue can heal on and heal in the new host for a certain time and can exert a favorable effect on the new formation of tissue. But the transplant itself does not take part in the new formation of tissue—it breaks down and is replaced by tissue belonging to the body. Without doubt it is possible that a living homotransplant can heal in temporarily even for some time. The durability of this transplant in its new host depends on many internal and external factors. A certain "organ hunger" of the new host can prolong the life of the homotransplant but ultimately the living homotransplant still remains only a parasite in the new host, from whom it can take over nutrient substances.

Gohrbandt's view is opposed to that of Fromme, who holds possible a continuance of life of a homotransplant in the transference of younger cells which are more capable of division.

Autografts and homografts of brown fat, abdominal fat, and omentum along with various other types of tissues taken from rabbits were studied by Williams (53) (1953). The transparent chamber technique was used mainly although it was supplemented by subcutaneous transplantation and intra-abdominal grafting followed by sectioning and staining to check on the effect of different environments.

In omental grafts *it was clearly demonstrated that vascular fragments and portions of the original capillary plexus survived and contributed to the new vascular supply, as well as did vessels invading from the host region.**

The autografts of brown fat survived but not the homografts. Brown fat cells obtained from the neck strikingly resembled cells from the zona fasciculata of the adrenal gland. No detailed studies of this tissue after grafting to chambers were made.

Autogenous abdominal fat survived in chambers. It increased and decreased in amount or disappeared entirely sometimes to reappear at a later date. Fat in some cells became temporarily crystallized. In some chambers fat appeared in the connective tissue sometimes essentially replacing it. "*In these cases there was little doubt that fat cells developed from connective tissue cells.*" Williams made no homografts of abdominal fat.

Eastlick of the State College of Washington reports that various aspects of the origin and development of "adipose tissue" in avian and mammalian embryos were investigated in his laboratory (55). Recently (56) he has tried to transplant small (approximately 0.5 mm.) pieces of embryonic "brown fat" and "white depot adipose tissue" in the lateral body wall of 2- to 4-day-old chick embryos. To date no positive results have been obtained. Eastlick says: "Perhaps the relatively small number of cells present in the implanted pieces is a handicap to its becoming incorporated in host tissues, or the relatively "loose" organization of depot fat tissue may not permit the implant to become vascularized before it is absorbed" (57).

Cameron and Malik (58) (1954) state: "Despite its bulk and ubiquity we do not know for certain whether adipose tissue can regenerate after injury, how it grows and what really happens when it undergoes necrosis. Even its functions are not altogether clear."

In their experiments on rats the abdominal cavity was opened and the right testicular omentum was withdrawn. An area of omentum was frozen by carbon dioxide snow for two minutes. The tissue was then returned to the cavity as a pedicled autograft. The rats were sacrificed at 24, 48, 72, and 120 hours and every other week up to 12 weeks postoperatively. Other experiments were performed in which rats were operated on and their testicular omentum frozen, but colchicine was given 6 hours before the animals were sacrificed.

In 24 hours the frozen region is distinguished by its cellularity, capillary congestion and hemorrhage. There is no clear evidence that fully-formed mature fat cells are able to divide by mitosis and form daughter cells.

The experiments show that a short period of freezing injures *fat cells* in such a way that they degenerate and perhaps become peculiarly vulnerable to phagocytic attack, breaking down and liberating their contents which fuse to form

* Similar findings were noted by the author in autogenous human fat grafts taken from the abdomen (54).

oil droplets of a larger size. The whole process results in oil cysts

As stated by Hausberger (59) many biologists agree that adipose tissue may develop from connective tissue cells as well as from lipoblasts. In his recent (1955) experimental work the immature testicular fat bodies of five-day-old rats were transplanted subcutaneously into the abdominal wall The fat body consists, at the time of birth and several days thereafter of mesenchyme-like fat-free cells indistinguishable from immature connective tissue cells (Fat deposition begins on about the seventh day )

In one group of rats the total fat body was transplanted and developed into a lipoma-like node consisting of mature adipose tissue of normal composition In the two other groups the fat body was divided into two parts. The transplanted pieces developed into mature adipose tissue nodes showing the same weight ratios as the original donor material.

To Hausberger these experiments indicated that the development of the final amount of immature adipose tissue depends, among other factors on the amount of immature adipose tissue transplanted and that the young mesenchyme-like adipose-tissue cells are cells with special potentialities.

As reported by Lever (60) brown adipose tissue in the normal starved and adrenalectomized rat was studied by both light and electron microscope. As judged by both methods the mitochondria lie between the fat droplets and range from 0.5 to 1 $\mu$ in cross sectional diameter The majority of mitochondria contain bilaminar internal cristae with an intercristal matrix substance Mitochondria may contain small quantities of an intensely osmiophilic material and bodies intermediate in appearance between lipid droplets and mitochondria are observed Mitochondrial limiting membranes are often deficient particularly at points of contact between mitochondria and lipid droplet which lie freely within the cytoplasm

Following starvation and adrenalectomy there is a reduction in total lipid and droplet size and the number of mitochondria is markedly increased All these changes are more pronounced and of more rapid onset in starvation and internal disorganization of mitochondria can occur if this is prolonged

## SUMMARY COMMENT ON TRANSPLANTATION OF FAT GRAFTS IN ANIMALS

A review of the literature on the transplantation of free fat grafts in animals from Bartels in 1908 to Neuhof in 1923 demonstrates that two schools of thought had become established regarding the behavior of the fat cells in autogenous grafts.

All investigators noted the early breakdown of fat cells the formation of cavities containing free fat, and *the constant presence of large host histocytes which contained fatty material* Some believed that these host histocytes were in process of taking on lipid and becoming the new fat cells which eventually were destined to replace all adipose cells in the graft. Others held that these large host cells merely acted as scavengers which removed the free fat from broken down adipose cells and that some of the graft fat cells survived these surviving fat cells constituted all fatty tissue present in the transplantation site after the host tissue reaction to the graft finally subsided.

Most investigators agreed that apparently normal adipose tissue was usually present in the recipient area eight months and longer after the transplantation of autogenous grafts They disagreed, however regarding the host or graft origin of the fatty tissue.

That autogenous fat grafts became reduced in size following transfer and that the grafts were sometimes largely or entirely replaced by host connective tissue were also observed by various authorities.

There was general agreement among observers concerning the behavior of fresh homogenous fat grafts these cross transplants were completely replaced by host connective tissue in most instances. Alternately some research workers noted limited but definite new adipose tissue arising from host cells that took on fat and became characteristic fat cells (Neuhof and others)

The literature on the transplantation of free fat grafts in animals from 1923 until the present time (1950) has not presented many important new contributions regarding the behavior of the ubiquitous fat cell in free adipose graft Investigators either have supported the host tissue replacement theory or have suggested that some of the adipose cells in free graft remain viable

and that it is these cells which constitute the adipose tissue that finally remains in the area of transplantation (the cell survival theory)

Evidence was presented indicating that autogenous embryonal transplants or autogenous grafts from very young animals may both survive and proliferate (Hausberger) The majority of investigators noted that the cells in fresh homogenous adipose grafts failed to survive transplantation and that homogenous grafts were replaced by connective tissue rather than adipose tissue.

Of particular interest regarding fat storage are the observations made by the Clarks (44) When single fat cells were held under observation in chambers inserted in the ears of rabbits they were seen to undergo cyclic changes involving accumulation of fat in small droplets, coalescence to form a single large droplet, subsequent decrease in size of this droplet and beginning repetition of the process. The cycles may be relatively short in some cases and perhaps longer in others. How often they are repeated by individual cells is unknown, so the assumption that fat cells are short lived is unwarranted. Dividing fat cells are scarcely ever seen. The same can be said of dying ones.

As described previously fat in storage is constantly undergoing change the old fat being removed from the cell and new fat, deposited The dietary fat stored in the cells of most animals varies within narrow limits, depending on the animal's food fat. Fat synthesized from carbohydrates and proteins however is probably constant for a given animal.

Fat tends to be deposited in adipose cells in regions where the circulation is sluggish Lipid is removed from cells where the blood circulation is rapid. Vitamin E and a fat rich diet lead to fat storage vitamin E deficiency and a fat rich diet are associated with minimal fat storage or an actual loss of stored fat.

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CHAPTER 7

# Transplantation of Fat (Continued)

LYNDON A PEER

## I EARLY TRANSPLANTATION OF FAT IN MAN

The first free transplantation of fat in man was possibly accomplished by Van der Meulen (1) (1889) who performed an operation suggested by Talma of Utrecht. This procedure consisted of grafting epiploon (omentum) between the liver and the diaphragm. Some adipose tissue was probably included with the omentum but Van der Meulen was more concerned with survival of the flat mesothelial peritoneal cells on the surface of the omentum than with the behavior of the underlying fat cells. Subsequently (1897) Braun and Bennett (2) closed a gastric defect from perforating ulcer with omentum sutured around the wound.

### REVIEW OF LITERATURE THROUGH 1923

Neuber (3) in 1893 was the first investigator actually to transplant fat grafts in man as separate adipose tissue and observe the behavior of his transplants. At the twenty-third Congress of the German Surgical Society Neuber stated that he had used small pieces of autogenous fat to fill out depressed scars with underlying loss of bone. One patient had a deep funnel-shaped cicatrix at the lower margin of the orbital cavity as a result of a tuberculous ostitis in childhood. Neuber detached the skin from the bone and inserted a segment of fat taken from the upper arm into the bony cavity. The adipose tissue graft was only of sufficient size to fill the depression adequately and the transplant healed in with excellent cosmetic result. He reported failures when large adipose grafts were used and concluded that pieces of fat which exceed the size of a bean or an almond do not heal in; *the smaller the piece the more certain is the result*

*In 1895 Czerny (4) reported his celebrated case* in which he had carried out successful autotransplantation of a large lipoma with its capsule *from the patient's lumbar region The lipoma* was used in replacement of a breast which had been removed because of chronic cystic mastitis. After a year the reconstructed breast was well formed but was slightly smaller and darker than the normal breast.

The following year (1896) Silex (5) described good cosmetic results following the use of free autogenous fat grafts from the abdomen in treating depressed skin scars after tuberculosis of the orbital margins.

Correction in a case of a deeply depressed scar adhering to the bone at the orbital margin was described by Zoege-Manteuffel (6) of Dorpat in 1896. He stated that he did not know whether the procedure had previously been practiced for he lacked time to search the literature on the question. A piece of fat from the cheek was partly freed from its connections so that it could be shoved as a padding over the osseous scar The pedicled fat flap was fixed with catgut sutures and the skin closed with silk suture The ectropion disappeared Smooth healing occurred with satisfactory result.

In discussing the treatment of hollow wound with rigid walls (bone cavities) Neuber (7) in *1896 reported that large fat transplant did not* heal in because suppuration occurred the wound opened and the fat floated out

Barraquer (8) of Barcelona in 1901 conceived the idea of transplanting fat in the cavity resulting from enucleation of the ocular bulb. Tentatively the procedure was completely successful.

Von Bramann (9) (1902) described a facial plasty in which he removed fat from the cheek and transplanted it in another location with a satisfactory result.

In 1903 Axenfeld (10) of Freiburg reported on fatty tissue transplanted to repair adherent osseous scars at the orbital margin. Silex in 1896 had stated that the transplantation of abdominal fat grafts was a procedure often used at the Axenfeld Clinic.

In separate accounts before the *Sociedad oftalmológica mexicana* in March 1903 López and Vélez (11) presented their experience in replacing enucleation of the eye. López performed 18 operations in which autografts from the gluteal region were used in the scleral cavity following exenteration, or in Tenon's capsule following enucleation with good adhesion. In Vélez's six patients the fat implant from the gluteal region adhered readily and obtained good nutrition. They used no more fat than necessary. The implants were well tolerated and gave good support for a prosthesis. They laid special value on the importance of never implanting more fat than exactly necessary to fill the cavity. Furthermore they recommended suturing with silk and providing meticulous asepsis.

In Schanz's patient (1904 1907) an osseous ankylosis of the humero-ulnar part of the elbow was made movable by chiseling out the split joint and laying in a subcutaneous pedicled fat transplant. The fat transplant, taken from the inner side of the upper arm with a broad attachment, was turned so that it filled the extensive joint fissure. Motion after closure of the wound was painless. Side motion was complete and extension of joint motion was only slightly restricted. Three months postoperatively the arm could be used in daily activity. Schanz considered fat an excellent choice for interposition between raw osseous surfaces, with prospect of favorable results (12).

In a woman with prolonged osteomyelitis Chaput (13) in 1904 opened the medullary canal of the tibia and dug an enormous cavity in the upper epiphysis which he filled with a piece of fat from the patient's abdominal wall. Cure was maintained up to 1912. At this time she had a small abscess, then a fistula below the fat graft of 1904. In making a new cavity he confirmed that the adipose tissue was healthy; the fat appeared white and sclerosed. There was a little suppuration and perhaps partial elimination of the graft, but meanwhile cure was obtained. Radiographically the old cavity of 1904 appeared to have diminished in volume about one-half. The tibia was hyperostosed and the fibula atrophied.

Lexer stated later in his book (1919) that in 1905 he had employed small free fat in ankylosis of the joints in the finger, the jaw and the elbow joint (14).

Murphy (15) (1905) emphasized that early free unrestricted motion is necessary in the use of a flap of fatty tissue, muscle or aponeurosis from the vicinity. In a patient with ankylosis of the hip application of a flap of skin, superficial fat and fascia lata from the hip and thigh resulted in the establishment of free motion of normal extent and the disappearance of pain. In another instance of involvement of the knee a transplant of fascia lata and adipose tissue with a broad pedicle above the joint level was turned in between the bones; the patient was able to walk.

In a comprehensive article published in 1906 Hoffa (16) reviewed the literature on surgical intervention in ankylosed joints and included a number of cases in which pedicled fat grafts or [free] fat-fascia grafts were interposed after resection. Some cases yielded good results, whereas others showed less favorable progress. Several cases of such ankylosis originated in gonorrhea, acute joint rheumatism or tuberculosis. Hoffa reported that he had obtained mobilization in jaw joints, shoulder joint, elbows and joints of the hand, a satisfactory outcome in the hip joint and some motion in the joint between the patella and femur.

Henle (17) reported to the German Congress of Surgeons in 1906 the results obtained from applying fat as a covering for detached nerves. In one instance he utilized free transplanted fat from the abdomen and another time a pedicled fat flap from the neighborhood of the nerve in a patient with ulnaris paresis. Observations of the patients extended only a short time so that he could not pass judgment on the permanent result.

Before the Heidelberg Ophthalmic Society in 1908 Bartels (18) of Strassburg narrated how he had been led to shove a large piece of fat from the abdomen or gluteal region or thigh into Tenon's capsule cavity after enucleation of the bulb Over the transplanted fat an artificial eye was well tolerated Three years later the cosmetic results were still present.

Verderame (19) in 1909 summed up the ten years experience with autogenous fat transplantation in ocular surgery at the Axenfeld Clinic *He noted that fat grafts became reduced in size following transplantation and advised the use of a larger transplant than seemed necessary to fill the defect*

In 1909 Lexer (20) presented a patient who had had a deep depression of the region of the malar bone and infraorbital margin. A piece of fat 12 cm. long and 2 fingers wide taken from the abdomen, healed in under the loosened skin and gave an excellent result. This, Lexer believed was the first case of extensive fat plasty applied for support (21) He also reported that he had been successful in building out receding chin (bird-face) with a similar procedure (20)

Marx (1910) reported a case in which the patient had had frontal sinusitis. The frontal sinus wall had been resected because of suppuration. After three months the granulations and wound edges having been freshened a fat graft from the thigh was laid in the wound cavity and the skin sutured over it. After some days the edges of the wound were firmly adhered When the patient was seen a year later the result of the operation was excellent only a superficial depression being present. Marx recommended placement of as much fat as possible in the wound cavity (22)

A child had cutaneous scars adherent to bone at the infraorbital margins and ectropion of both lids. A fat graft from the thigh was introduced under the skin at the infraorbital margin and resulted in firm padding The ectropion was relieved and the final result excellent (23)

In an exceptionally large experience (for the year 1910) with 37 cases of enucleation of the eye Lauber (24) of the Eye Clinic in Vienna implanted autogenous subcutaneous fatty tissue from the abdominal wall in the defects In six patients the fat necrosed and was eliminated. He noted clinically that the volume of fat diminished slightly during the first two to three months following transfer After that it did not alter in mass enough remained so that it provided support for the prosthesis As the implanted fat felt firmer it was thought that it was partly replaced or penetrated by connective tissue

Bier (25) (1910) of Berlin remarked at a German Surgical Congress that he very frequently transplanted adipose tissue for cosmetic purposes with very good results. He treated facial hemiatrophy in a boy by padding the defect with strips of a lipoma presumably from the same patient. At the end of three weeks observation the result was excellent but Bier did not determine whether the implant held permanently

In 1911 Lexer (26) reported transplantation of a large autogenous fat graft, 12 cm. long, 3 fingers wide and 2 fingers thick, taken from beneath the breast in the subcutaneous bed of a large facial defect in the region of the orbital cavity There was no shrinkage and the facial defect was filled with a soft even mass that reestablished natural contour lines This successful case led him to widen the field of application of autogenous fat plasty as in forehead depressions, saddle nose the sunken cheek and in the submental region. Implanted fatty tissue held well in correcting osseous cicatrix after depression fracture Clinically the deposited fat mass shrunk slightly and became somewhat firmer but the transplants held in all of his patients. It is possible Lexer says, that the crushing and tearing of fat grafts during removal cause larger transplants to fall prey to necrosis in the healing process and to disappear *He emphasized careful preparation of the site of implantation and immediate transfer of the removed piece of fat*

In a young woman with a severe facial deformity following osteomyelitis of the maxilla Morestin (27) (1911) excised the cicatrices and inserted a strip of autogenous fatty tissue taken from the buttock. He considered application of the fat graft to be the method of choice whenever it is necessary to fill a depression or an aseptic cavity

Stucla (28) (1911) cited a personal case of successful implantation of fat to pad a vast cicatrix resulting from noma (stomatonoma) of the cheek, and a similar good result from a free fat graft in a deficient female breast

At a meeting of the German *Gesellschaft für Laryngologie* in 1911 Brunings (29) made known

his new method of nasal plasty which consisted of injecting with a syringe under the skin autogenous fatty tissue cut into small cubes. The immediate results in four patients were excellent but in a communication a few months later he expressed less satisfaction with the late results and foresaw progressive resorption of the prosthetic mass.

Before the *Société de chirurgie de Paris* in 1911 Tuffier (30) showed a series of sections of a fat graft taken from the abdominal wall of an obese woman and introduced into the extra pleural space as an autograft. The transplant remained *in situ* for four months. The sections consisted of the fat graft and pulmonary tissue which was continuous with a very dense lamellar fibrous tissue rich in cells and containing numerous dilated vessels engorged with red cells at certain points. This fibrous tissue emitted prolongations of dense fatty tissue In places it resembled islands of newly formed fat becoming part of the pleural fibrous tissue but different from the pleural tissue especially in the absence of embryonal infiltration. (This report by Tuffier is the earliest reference found in which an autogenous human fat graft was removed and examined microscopically )

Also in 1911 before the International Surgical Congress at Brussels Tuffier (31) described a procedure for grafting large masses of fat between the mobilized pleura and the ribs in treatment of pulmonary conditions. If without thoracic resection the wall of the pleuropulmonary cavity could be pressed down, and the intercostal muscles detached and pushed back from the wall of the cavity the space thus created was filled with either a fresh fat autograft or a preserved fat "heterograft. * The results clinically were satisfactory

Tuffier (32) (1911) also applied an autograft of adipose tissue from the thigh to an osseous cavity in the femur produced by osteomyelitis of 15 years duration, and a homograft of omental tissue (mesothelium and fat) to a resected elbow joint. He pointed out that the fat graft obstructs the cavity but does not prevent continuation of the infectious process it can be alive and yet a new abscess can form in the region

* Tuffier means homogenous not heterogenous The French and other Continental writers sometimes use heterogenous to denote any graft other than the patient s own tissue

Nélaton (1911) transplanted an autogenous fat graft in an osteomyelitic cavity with immediate perfect result but suppuration and elimination of the graft soon occurred Ombrédanne obtained primary union in certain cases in others healing occurred only after a more or less long delay In Walther s case of a fat graft from the thigh implanted in an osteomyelitic cavity, a good result at first was followed by ulceration and elimination of a small part of the graft (33)

In a thesis (1911) on the treatment of large osseous cavities due to osteomyelitis by fat graft Parent (34) described Chaput s technique and pointed out the advantages of the procedure and indications for its use Cases of Chaput and one of Souligoux are cited along with a personal case. Parent s patient (on Chaput s service) had had several previous surgical interventions. Trepanation of the tibia produced an osseous cavity of the leg with loss of substance After the ulceration had been incised an adipose graft larger than the size of the cavity was placed in the wound Despite expulsion of a fragment of the graft the result was favorable with rapid cicatrization.

Röpke (35) (1911) reported two cases of ankylosis of the joint—the elbow joint and a finger joint—which were treated by interposition of free autogenous fatty tissue The results were favorable

In a young man with neurological symptoms following head injury Rehn (36) (1912) after freeing cerebral adhesions laid on the surface of the brain a fat transplant taken from the upper arm, thus protecting the nerve substance against osseous contact. The fat transplant covered the scar in the cerebral cortex and the surrounding dura its projecting parts were shoved under the edge of the vault of the cranium. Healing was smooth the patient suffered no more attacks and no further pain.

Klapp (37) (1912) reported on the transfer of large autogenous fat grafts from the region of the major trochanter in two patients to correct a defect caused by chronic interstitial mastitis of the breast. After three months the transplant was still in place in both patients with an excellent cosmetic result. The graft was somewhat shrunken but solid In one patient the transplant was the size of two fists.

In the same year at the University Surgical Clinic of Berlin Klapp (38) as cited by Zipper

(39) operated on two patients in whom the mammary gland was completely removed because of benign neoplasm and filled the defects with free autografts of subcutaneous fatty tissue. These two operations according to Zipper were the first in which the whole mammary glands were replaced by means of such a procedure Zipper made a test excision of the free fat autotransplant four months after transfer to study its fate * In order to reach the transplant he had to cut through "a sort of capsule" Microscopically he found "living normal fatty tissue with wide connective tissue septa rich in leukocytes. The transplanted subcutaneous fatty tissue as viewed by Zipper had been maintained as such and was surrounded by a connective tissue covering formed of stroma.

Makkas (40) (1912) successfully implanted a free fat graft from the gluteal region in a cavity due to tuberculosis in the head of a metatarsus. Three months later there was complete motion in the joint without pain. In another patient a walnut-sized transplant of fat from the upper leg was used to fill a defect in the tibia also due to tuberculosis of the knee joint In a third patient severe suppuration took place after implantation of fat from the gluteal region in a cavity but the fat graft was not extruded

Hesse (41) (1912) removed a pathologically changed ivory hard mass from a young man with osteomyelitis of the left tibia. A free fat graft from the gluteal region was transplanted into the large medullary cavity with closure of the periosteum and skin There was no reaction pain disappeared palpation gave normal finding Roentgenographically there appeared a deep depression in the bone at the site of chiseling

In November 1912 Chaput (42) presented evidence indicating that a fat graft implanted in the epiphysis of a woman patient in 1904 was healthy The fat grossly appeared white and sclerosed and there was a little suppuration with perhaps partial elimination of the graft Complete clinical cure however was obtained. Radiographically the graft was later reduced in volume it was vertically about half of its former size The tibia was hyperostosed and the fibula was atrophied

Chaput (43) (also in 1912) interposed a free fat graft in ankylosis of the elbow resulting in satisfactory mobility and articular solidity

In his detailed and comprehensive contribution to orthopedic surgery in 1912, Murphy (44) told of using a transplant of autogenous fascia lata and trochanteric bursa with the overlying fat from the hip transposing it on a pedicle into the ankylosed knee joint. This resulted in considerable motion in the joint

In another instance a flap from the aponeurosis of the supinator longus and a flap from the fascia and fat on the inner side of the elbow with the bases of the flaps directed upward were applied to the elbow joint with perfect functional result

After a thorough review of the literature on transplantation of omentum, Lutz (45) (1913) expressed the view that in free fat transplants "we possess a method which merits wide application in all kinds of different conditions especially in cosmetic surgery The procedure is easily carried out and with careful foresight guarantees certain success. A fat graft is living tissue"

On the other hand Smirnoff (46) (1913) discouraged the use of fat alone as a transplant although he reported the successful repair of a dural defect with autoplastic fat.

In 1913 Morestin (47) referred to a series of remarkable successes in correcting certain facial deformities. He held that fat grafts have innumerable indications in reparative surgery such as repair of loss of cranial substance all depressions left by traumatism surgical interventions or pathologic processes in the region of the forehead cheek bones jaws or parotid region. In eight cases of fat grafts he had only two failures

In Morestin's case of a young man who sustained a blow on the left side of the face at the cheek bone and anterior part of the cheek suppuration and elimination of sequestra supervened with formidable hemorrhage After débridement of the fistula and excavation, the wound cicatrized Later sclerosed tissue was removed and a rather large piece of gluteal fat was placed in the facial wound Union was perfect and the cosmetic appearance very satisfactory (48)

In Perimoff's patient on the 17th postoperative day when the skin of the wound over a free fat graft had to be freshened the fat padding on

* This represents the second earliest reference to the removal and microscopic study of an autogenous fat graft in humans

the face appeared to be unchanged and did not bleed when the superficial layers were removed (49)

As reported by Galpern (50) (1913) a radical operation was undertaken on a patient with empyema of the frontal sinus. After seven months the patient returned to the hospital with a severe depression in the operated region. At operation the soft part was detached from the bone, producing a cavity into which a piece of fat was transplanted from the gluteal region. A smooth postoperative course was followed by an excellent cosmetic result with no change after three months.

In an address before the Western Surgical Association in 1913 McArthur (51) expressed the opinion that for existence and maintenance fatty tissue requires little more than plasma and asepsis under body conditions and it can therefore be transplanted as a free graft in almost any bulk provided these conditions are maintained In his hands cosmetic filling out of the deformity from partial resection of the lower jaw gave most gratifying results. He used autogenous fat-fascia to protect the musculospiral nerve just released from the callus of a humeral fracture and to cover a nerve trunk just sutured after resection of a neurofibroma.

A transplant of fat, thick as the thumb and large as the hand taken from the thigh was used by Rehn (52) (1913) to cover the brain in a head wound with splintered bone The osseous defect was simultaneously replaced by a pedicled periosteal bone flap Recovery of the patient was satisfactory

Rehn recommended the necessity of subjecting all cases to careful examination and of utilizing only pure, typical cases of partial and general traumatic epilepsy He held that only an observation of three to five years after operation justified speaking of a patient as cured

At the Medical Society of Magdeburg in April 1913, Wendel (53) presented a woman who had had hemiparesis associated with head pain and dizziness. After removal of a tumor (endothelioma) with a decomposing cyst, there was a large defect between the dura and upper surface of the brain. To make wound closure possible a free graft of fascia lata from the thigh was fixed in the dural defect and the remaining space was filled with a piece of fat from the same thigh The soft mass of fat filled the irregular space well The paresis rapidly retrogressed There was no fistula and the transplants firmly and smoothly healed in No disturbing effect resulted

Good results were obtained by Hayward (54) (1913) in complete and partial restoration of breast by fat transplantation. Autogenous grafts of fat taken from the thigh were transferred into the breasts, twice on account of chronic interstitial mastitis and twice for adenofibroma

On the basis of clinical and experimental observations Röpke (55) (1913) carried out free fat transplantation to fill out surgical osseous cavities and introduced the method in joint surgery The procedure was used in ten joints in nine patients involving the finger hand elbow shoulder hip and knee joints, and once in a synostotic finger joint. Free fatty tissue was also transplanted into a cavity after resection of the joint and removal of diseased tissue due to tuberculosis. Primary healing occurred in all patients. These different types of involvement are demonstrated by case reports In Röpke's judgment fat represents an extraordinary transplantation material. Removal and implantation must be rapid and the implant must be protected from injury

Klopfer (56) (1913) gave the histories of eight cases of free fat transplantation in osseous cavities from osteomyelitis of the tibia empyema sinus frontalis osteomyelitis radii femoris and tuberculosis. Permanent results could not be determined In aseptic osseous cavities healing of the implant, as stated by Klopfer is the rule In infected cavities the transplant of fat can heal in yet the course of healing is delayed through fistula formation.

Lawrowa (57) (1913) referred to two cases in which fatty tissue was transplanted into osseous cavities, due to osteomyelitis. In the first patient with chronic osteomyelitis primary healing of the wound occurred and the scar was perfect after four months. In the second patient the osteomyelitis was associated with fistulas and the osseous cavity included at least two-thirds of the humerus. Partial suppuration took place but healing occurred after three months

Writing in 1913 Estor and Étienne (58) stated that since Chaput utilized a fat graft in an osteomyelitic cavity for the first time in 1904 22 new cases had been published In three patients in their care with involvement of the tibia such

a procedure resulted in near or complete cures. Cases may be considered of doubtful success when oily drainage is observed. Even though the method is reserved for chronic osteomyelitis, all these cases are equally favorable The cases in which the results have the greatest chance of being successful are those in which the cavity is small. Lator and Étienne *preferred to use autografts* they emphasized asepsis and drainage of the wound for 48 hours to avoid the occurrence of hematoma The graft should be at least a third or a half again as large as the cavity to be obliterated They considered disinfection of the cavity the most delicate point of the operation. In their opinion, obliterating osteomyelitic cavities with fat grafts merits continuation from the clinical and experimental viewpoints.

Lambret (59) (1913) reported success with the *application of fatty tissue grafts in a bronchiectatic* cavity of the apex of the lung

Lemormant (60) (1913) discussed extra pleural pneumothorax for cavernous tuberculosis He described a procedure consisting of separation of the parietal pleura and filling the cavity thus created with an aseptic and nonabsorbable mass either a fat graft or inorganic sealing substance He reviewed 20 cases in the literature in which the benignity of the operation was established and in which not a single death occurred The only outstanding incident was the persistence of the septic wound in a patient with gangrene To Lemormant at the time of writing the facts were still too few to decide if preference should be given to organic filling with a fat graft or to the use of inorganic implants.

Chaput (61) (1913) presented an obese woman on whom he had operated the previous year for *an enormous umbilical hernia. A* large rolled adipose graft 12 to 15 cm. long by 3 to 4 cm. thick taken from the abdomen was placed in the peritoneum behind the abdominal wall as a *support for the hernia The end of the hernial* ring was sutured to the graft. After a long convalescence reduction of the hernia was perfect No impulsion was seen at the level of the umbilical ring

In one case of gall-bladder removal reported by Hilse (62) in 1913 application of a free omental graft and subcutaneous fatty tissue stopped the bleeding in a few minutes In another patient a free graft of rich subcutaneous fatty tissue stopped bleeding of the liver in an operation on the gall bladder In both patients the course of healing was normal.

Polenow (or Poljenoff) and Ladygin (Lodygin) (63) in 1913 confirmed their animal experiments (see Chapter 6 page 181) by three clinical cases of wounds in humans. In a kidney wound at the hilus after unsuccessful suture implantation of a piece of perirenal fatty tissue produced direct control of the hemorrhage A piece of subcutaneous fat was transplanted to a wound in the liver with the same prompt result. Suturing of a piece of fat in a lung wound stopped bleeding at once

Eden and Rehn (64) (1914) reported four *cases of paralyses treated with neurolysis and* subsequent covering with fat In two cases complete cure had not yet occurred nerve and muscle not being back to normal In one instance *there was slight atrophy of the thenar eminences* of two fingers In two patients after the adherent tendons had been freed they were covered with an autogenous fat graft. The transplants healed in without reaction in spite of being embedded in cicatricial callus. The fat graft later could be palpated beneath the skin. There was no reaction from fatty tissue applied for bridging a mucous-membrane defect of the urethra After the urinary fistula had closed urination was normal. The fat graft was not extruded

As stated by Lexer (65) in 1914 he carried out the first large free fat transplantation After three years the first patient showed the result obtained after padding a severe depression of the zygomatic arch caused by fracture His first operation with free fat transplants was made on the elbow in 1906 Since then he has tested the procedure on the finger hand elbow and foot *joints. He introduced the procedure in treat*ment of ankyloses in joint surgery and in various other repairs.

In a young woman Morestin (66) had im*planted an adipose graft to correct a* cavity produced by freeing a depressed adherent cicatrix after osteomyelitis of part of the jaw The graft having been taken slightly larger than was necessary he excised *a part at a secondary* operation * thus restoring the symmetry At the Congress of the International Society of Surgery (1914) Morestin gave some histologic details The

* This is the third reference found describing the histologic examination of an autogenous fat graft The first was by Tuffier in 1911

center of the fat mass, presenting a clear homogeneous yellow color was very friable and was dissociated into a sort of pulp In the rose-gray periphery were interspersed a multitude of small newly formed vessels In this zone fat was no longer present but young connective tissue was in continuity with cellular tissue of the neighborhood The fat mass had served as a substratum and its tolerance was only a transitory state Morestin commented "not a parcel of adipose tissue was really graft. The bundle of fat disappeared without leaving the least vestige after having been replaced by connective tissue. Conclusively the fat graft is transformed into connective tissue if the result is stable

*Morestin believed that a fat graft could be taken from either the same subject or another subject but preferably from the patient himself* because adipose tissue is poor in vessels and almost inert It is one of the tissues which can be most easily transferred from one region to another Its torpid vitality and its weak demands become advantages.

In a patient in whom parietal bone and some lacerated brain substance had been removed Binnie (67) (1914) implanted fat from the abdominal wall to fill the defect resulting in good recovery He also obtained a gratifying result from the use of fat to fill a cavity left by removal of a metacarpal tuberculous focus. It was his belief that in many instances it is the connective tissue basis of the fat which is of value in the transplant. Binnie suggested the use of free flaps of fat and fascia in arthroplasty

Devine (68) (1914) of Melbourne based his clinical work with free fat and fascia transplantation in the treatment of ankylosed joints and diseases of bone on the experimental work by Makkas at Garré s Clinic in Bonn. In some patients pieces of fat with underlying fascia larger than a hen s egg were transplanted with complete healing of the graft. Devine considered autogenous implants the most favorable type of graft.

In one patient with a fairly large sequestrum in the lower end of the femur fat from the gluteal region was pressed into the cavity produced by excision of the sinuses The wound healed by first intention. Most of the fat was replaced by bone leaving residual fat oily fluid broke out through the old scar 12 months later

After unsatisfactory use of a pedicled flap for arthroplasty of the shoulder joint a large piece of fascia lata with one-half inch of fat was used to enclose a new head of the humerus and a part of the shaft, with complete healing Movement of the joint was perfect and painless 18 months later

A free fat graft ("flap") used in arthritic ankylosis of the hip joint resulted in flexion 12 months later

In a woman with ankyloses of the jaw due to bilateral parotid abscess, two teeth had been removed to allow the taking of nourishment. The lower jaw was undeveloped there was no temporomaxillary joint and no sigmoid notch. The only representation of a temporomaxillary joint was a free serrate line. Interposed grafts ("flaps") of fascia lata and fat from the leg were used for reconstructing the temporomaxillary joint.* Three months later there was almost perfect, complete and painless movement of the jaw

Free fat transplantation as expressed by Devine can be relied upon to do all that is claimed for a pedicled graft. A stronger and earlier functioning joint results with free transplantation rather than with a pedicled flap Fat merits wider surgical use, in his opinion.

Ferran (69) (1914) reported a case of osteitis of the tibia following a complicated fracture, which was treated with a fat implant. After reduction of the fracture suppuration occurred and a fistula formed The fat autotransplant from the thigh was placed in the osseous cavity of the tibia Subsequently union of the wound was partial the fat implant was well tolerated and ultimately cure was perfect so that the patient could return to work

Röpke (70) (1914) reported two cases of joint ankylosis which were treated with interposition of free transplanted fatty tissue one of the elbow, the other of the finger The results were favorable

In a case of scar fixation of the brain to the skull as the cause of epilepsy and cephalalgia Wendel (71) (1915) described removal of the outer scar and resection extending to healthy dura to obtain access into the free intermeningeal space A free fat transplant taken from the thigh was applied with fascia outwardly In Wendel s

* Devine s transplants were free grafts although he refers to them as flaps

patients operative freeing of the brain produced rapid healing and improvement or cure of the epilepsy

In the hospitals of Düsseldorf in 1915 as reported by Döpfner (72) application of fatty tissue was practiced in neurolysis in partial and total nerve suture A rectangular fat flap with superficial fascia from neighboring skin if possible was freed and folded around the nerve trunk so that the fatty side lay toward the nerve The fascia lata was fastened above and below to paraneurotic connective tissue or to adjacent muscle or fascia. In partial nerve suture free pieces of fat fascia enveloped the nerve and in total nerve suture it was stretched under the nerve and along it

In 1915 Morestin (73) reported on a series of war wounded in whom grave deformities were either corrected by means of fat grafts or considerably ameliorated by such grafts. Nine cases were presented as examples showing how the fat graft can be useful in treating certain facial deformities resulting from loss of skeletal substance or soft parts. The "before and after" photographs show good results. At this time Morestin noted that fat transplantation had made considerable progress in reparative surgery

In Souligoux's case a captain had had his right eye and a part of the malar and zygomatic bone removed surgically A severely adherent cicatrix to the bone associated with slight ectropion remained. As the patient was affected psychically by the deformity Souligoux cut away the skin adhering to the deep tissues and inserted a large piece of fat taken from the patient's gluteal region. Healing was perfect and the deformity was completely effaced (74)

Mauclaire (75) reported to the *Société de Chirurgie* December 1 1915 an unpublished case in which there was functional inability of the extensor muscles of the digit Cicatricial adherence of the fleshy part to the skin was liberated by insertion of a fat graft.

Strandberg (76) (1915) referred to an interesting case of a girl in whom a pedicled abdominal skin and fat flap was transplanted to the dorsum of the hand where loss of skin had occurred through a burn The result was satisfactory for a time Then the patient took on an increase in abdominal fat following operation and the transplanted fat in the hand correspondingly increased in size

Free fat transplants taken from the subcutaneous tissue of the thigh were employed by Wrede (77) (1915) in three patients. Adipose replacement of the semilunar bone in fracture resulted in elimination of the symptoms. He also reported the implantation of a large fat graft in a mammoplasty after removal of a fibroadenoma which resulted in a fistula of ten months duration The fat implant became slightly shrunken but the cosmetic result eventually was excellent

Wrede also used a fat flap to establish movement in an ankylosed elbow joint. The operation restored normal motion completely

A case in which the patient had cancer of the penis necessitating cutting away part of the femoral vein was reported by Eloesser (78) (1915) A bit of fatty tissue removed from the wound in the groin was tacked over the opening in the vein by a fine silk stitch. At necropsy 12 days later the lumen of the vein was found to be free of clot and its walls smooth. The graft was firmly adherent and not necrotic Microscopically the fat cells in the transplant stained well although much leukocytic infiltration was present.*

Kolischer (79) (1915) of Chicago pointed out the usefulness of fat transplantation in pyelotomy in removing a calculus through the parenchyma of the kidney and to control hemorrhage in nephrotomy If sufficient perirenal fat is not available a free fat transplant taken from beneath the skin adjacent to the primary incision is recommended If a nephrotomized kidney is of soft consistency so that sutures threaten to cut or tear through the inadequate resistance of the renal tissue can be reinforced by the interposition of fat, which also has a hemostatic effect. In suprapubic prostatectomy a free transplant of fat may be used as a "live tampon" in the former site of the enlarged part of the prostate To quote "the transplantation of fat by using either a connected flap or a free transplant finds a field of great usefulness in genitourinary work

Kolischer's article in 1916 consists only of illustrations of fat transplants in kidney and bladder surgery These include fat transplant from the abdominal wall into a cavity resulting from removal of the prostate over traumatic rupture of the kidney attachment of fat for

* Eloesser gave the fourth report in the literature regarding the microscopic findings in an autogenous human fat graft

reinforcement of the pelvic wall and a fat flap drawn over a nephrotomy wound thus utilizing the hemostatic qualities of fat (80)

On a number of occasions Kolb (81) (1916) made use of fat transplants in nerve wounds In one patient with a wound of the thigh suture of the nervus peronaeus was executed some distance above the knee joint and a strip of fat laid around the area where the nerve was sutured. Primary healing occurred and function gradually returned in the nerve Paresthesia however developed later which on exposure of the nerve was found to be due to swelling This was caused by the fat transplant, lipomatous degeneration. The fat graft had taken on volume the individual flaps being enlarged The strips were still in place around the nerve suture but not adherent. The transplant weighing 30 gm. was removed and the disturbance in sensibility ceased shortly

Kanavel (82) (1916) presented the results from transplantation of free fat flaps from the abdominal wall or leg to prevent the development of scar tissue about cut tendons, about nerves and blood vessels and to restore mobility to scar tissues and joints. Where possible no sutures were used to retain the graft in position and care was exercised to prevent development of hematoma. Fat grafts were also transplanted into brain defects, into osseous defects and in contractures of the hand following infection. In 4 out of 32 patients there occurred secondary infection and loss of fat Kanavel believed the statement may be safely made that fat can be transplanted into any ordinary field with the assurance that it will not act as a foreign body *Clinically it would appear to live and become a part of the structure in which it is placed* The fat implant, he concluded persists for many months and probably years. He advised that fat should not be packed too tightly in the field in which it is placed. "The greatest value of fat transplantation is to be found in its use in plastic operations to restore mobility and remove disfigurement as a protection to prevent contracture about vessels and nerves and to prevent adhesions about tendons and joints.

In epilepsy after gunshot wounds of the skull Guleke (83) (1916) emphasized the importance of preventing firm adhesions of the brain and skin to the osseous skull covering and of filling out the defects in the brain so that no scar retraction occurs. This can be done satisfactorily by implantation of fat (Lexer) In larger defects Guleke used gluteal fat, which up to egg size heals in smoothly and is well tolerated The subcutaneous fat from the anterior surface of the tibia, which is transplanted with a bony piece is sufficient in superficial trough-like defects.

In more than fifty transplantations from the tibia, Guleke did not observe a single disturbance all healed smoothly In most patients the post operative epileptic seizures occurred during the first two weeks. Cures of epilepsy can be judged only after years. The patients in whom the scars were radically excised feel best. In Guleke's opinion, fat plasty should be carried out a half year after the injury at the earliest

In 1916 Vorschütz (84) covered the brain defects of three patients with transplanted fat in masses as large as a hen's egg The fat was removed in strips from the gluteal region rolled up and placed in the cavity Smooth healing took place in each case In two patients with injury associated with severe spasm of the lower extremities, immediately after the operation movement of the legs became easier and was no longer spastic Gradually further improvement was evident in the ability to move even though slowly

Vorschütz recommended an interval of 4 to 6 weeks for healing in of the fat implant before covering the osseous cavity usually without replacing the dura. He believed that in such circumstance the upper surface of the brain after such fat transplantation displayed a smooth regular appearance so that replacement of the dura became superfluous.

In the war casualties of 1916 crushing of the skull deep brain wounds and dural defects repaired immediately with autotransplants of free fat and fascia were reported by Kaeger (85) Deep destructive wounds involved the cerebrum and the parietal and occipital regions of the brain. In some patients free periosteal bone flaps were used to fill osseous defects The primary healing was good

In view of surgical findings and results in case histories Eisleb (86) (1916) concluded that free transplanted fatty tissue represents an excellent grafting material In all patients in whom it was transplanted in infected regions it healed in without reaction. The results in joint

function were also good To isolate cerebral cortex from the dura or skull cap in brain surgery fat proved to be a firm interposing material which healed without reaction. Fisleb gave histories of cases of ankylosis of the hand joints elbow joint hip joint osteomyelitis of the femur arthritis of the hip joints and so forth

Mathieu (87) (1916) presented before the *Société Médico-Chirurgicale militaire* an injured patient on whom a fat graft had been used to repair a loss of substance of the face leaving orbital deformity The fatty tissue taken from the buttocks filled out the bony framework of the cheek with a good cosmetic result. *Recognizing the inevitability of resorption of fatty tissue Mathieu affirmed the necessity of cutting the graft in much larger size than the loss of substance*

In a patient with comminuted fracture of the superior right maxilla with large loss of substance of the palate Pont (88) (1916) filled the depression with adipose tissue taken from the buttock The course of recovery was normal the constricture of the jaws under the usual treatment disappeared

Mauclaire (89) presented to the *Société de Chirurgie* in 1916 a patient on whom he had performed a fat autoplasty to fill a depression of the parotid gland Atrophy of the parotid gland of undetermined cause occurred in infancy A gluteal fatty autograft filling the depression was partly eliminated

In a patient who had sustained a fracture in both bones of the forearm as narrated by Schläpfer (90) (1916) bony union had taken place preventing motions of supination At the Surgical University Clinic at Leipzig the callus was excised and the radius was enveloped in a free fat-fascia transplant taken from the thigh Movement was apparently satisfactory after operation. A roentgenogram showed normal configuration of the bones of the forearm Some bone splinters appeared to have remained behind in the soft parts and were in the process of resorption.

At a German medical society session in 1916 Küttner (91) presented an officer in whom he had transplanted a large free fat flap from the thigh in an ankylosed knee joint following a wound. When the patient walked the side which had been affected was not distinguishable from the other side

Roper (92) (1917) of Jena referred to a case of a wound of the frontal bone eminence with torn margins of bone and dura Fascia lata taken from the thigh was transplanted on the dura with good healing but due to pains in the head a second operation was carried out (by R. Nieny) On both sides almost the whole frontal bone was lacking. After loosening of the scar and evacuation of serous fluid a fat graft was laid on the brain defect and two periosteal bone flaps from the parietal bone were applied and fixed primary healing taking place Gradually the patient showed psychic improvement but developed signs of a traumatic psychopathic constitution.

In war wounds of the skull (1917) Marburg and Ranzi (93) adopted the surgical method of treating epilepsy with implantations of fat. A large piece of fatty tissue from beneath the abdominal skin was inserted as a pliable buffer between the upper surface of the brain and the osseous parts. In a series of cases they were satisfied with the use of this method on patients in whom the epileptic seizures indicated operative intervention. In some patients an osseous covering was provided in the same operation. *On the whole however their surgical interventions gave them little satisfaction* In some instances after temporary improvement and absence of attacks, the epileptic seizures recurred In others it was not known whether they might not recur later

Siegel (94) (1917) presented a soldier in whom two defects in the skull from a shot wound were overlaid with a periosteum-covered bone flap from the tibia and padded with fat transplants from the lower leg

In 1917 Nieny (95) reported the case of a seaman wounded in the temple in whom a fat transplant from the thigh was introduced under the edges of the dural defect The bony defect contained a free parietal periosteal bone flap. The course of primary healing was smooth the patient being up in ten days but he succumbed to an intracranial hemorrhage on the 10th day postoperatively

At autopsy the bone covering was adhered to the edge in a small area but was detached from the fat implant for the most part The fat transplant was only partly adherent to the softened brain section and showed some connective tissue septa. The undersurface was overlaid with connective tissue The places where firm union with the softened brain part had occurred were covered with brownish masses in layer *Micro-*

*scopically the transplanted fat was well maintained everywhere* There was no increased connective tissue in places of disintegrated fat In Nieny's opinion, the procedure appeared justifiable showing how a fat transplant can heal in without degeneration and can prevent formation of callous scar on the upper surface of the brain Eventually the fat transplant has little tendency to form firm adhesion, especially with the healthy brain parts.*

Gross (96) (1917) reported a case in which the patient had a fistula following a war wound of the breast. There was delay in healing with rib cartilage necrosis. A fat flap from above the shoulder including muscle tissue was pushed into the lung cavity This resulted in smooth permanent healing after suppuration subsided

In a case of a typical Dupuytren s contracture of the finger, as narrated by Peiser (97) (1917) the tendons were unchanged Fat removed from the lower abdomen was laid over the palmar wound and fixed with fine silk sutures. At first marginal skin necrosis of the wound occurred. Then smooth aseptic healing of the transplant was observed over a period of four months. The fat padding gradually became a soft elastic cushion which was not adherent to either the skin or the tendons. Motion of the fingers was free. Peiser passed no permanent judgment on the outcome it was promising

In a case presented by Caforio (98) at the twenty-fifth *Congresso della Società italiana di Chirurgia* in March 1917 suppuration took place after fracture of the tibia in a wound at the middle third of the right leg leaving a cavity A large block of fat removed from the patient s buttock was fitted in the osseous cavity which showed no signs of closing Healing was by primary intention. At the end of forty days the patient was completely cured so that he could use the limb freely

Freund (99) (1917) of Strassburg applied two thick folding-door fat-fascia flaps from which the skin had been removed to cover a perineovaginal wound and to prevent recurrence of prolapse of the uterus. Smooth healing took place

After reporting on experimental transplants in the traumatized kidney and in pockets formed in the bladder wall of dogs, Koll (100) asserted that clinically he filled cavities made by enucleation of the prostate with fat graft. This he suggested, can be taken from the patient s abdomen or better still, from a dog (heterogenous) before the operation and preserved on ice He made no mention however of using such heterogenous grafts. He believed fat to be a valuable hemostatic following prostatectomy and in operations on the kidney

The fifth microscopic report in the literature

In Wagner's patient with a defect of the skull (1918) hydrocephalus internus of a lateral ventricle occurred Two large fat grafts taken from the abdomen of another patient and immersed in physiologic salt solution were used to fill in the cavity *The homotransplant healed without disturbance* The ventricular system reacted with slight meningeal symptoms in the first days but later no harmful influence on the brain was observed A year following the fat transplantation the patient was doing well A facial tick had diminished and there were no complaints (101)

In his excellent and very inclusive discussion of fatty tissue transplantation in 1910 * Lexer (102) reviewed his wide clinical experience in applying fat grafts for various purposes. An important factor in the hemostatic property of transplanted adipose tissue for parenchymatous hemorrhages is fresh agglutination to the bleeding wound surface

Lexer *frequently transplanted massive free autogenous fat grafts* for traumatic depressions and deformities of the facial skeleton—of the forehead cheek, jaw chin and orbital margins. After rhinoplasty it was often necessary to improve the form of the ala nasi or the tip with fatty tissue padding Lexer recommended that the incisions be made as invisible as possible by selection of the site so that the subsequent scar does not lie over the transplant. Clinically he applied fatty tissue for filling out an orbital cavity as replacement material in mammary deformities and for pendulous breasts Lexer obtained good permanent results in partial defect

* In this book Lexer describes his earlier clinical work with fat grafts much of which had not been published It is curious that he quoted other investigators regarding the behavior of fat cells but did not remove biopsies from his own transplants and examine them microscopically

Later surgeons have not been able to duplicate Lexer s good clinical results and most plastic surgeons despite the availability of antibiotics do not use massive free fat fascia or fat-dermal transplants extensively

of the breasts by using very large autogenous fat graft which in his hands were satisfactory although some late absorption did occur He also used free autogenous fat grafts and fascia fat graft and flaps to reestablish motion in ankylosis of the hip joint

Fatty tissue was also employed in osseous cavities (when healthy conditions prevailed) for filling out dead spaces for fistulous cavities due to empyema and lung fistula, in the surgical bed after thyroidectomy to replace the testicle and for cavities of single carpal or tarsal bones.

Fat grafts for enveloping nerve and tendon to prevent adhesion after tendolysis and neurolysis and after nerve and tendon suture were first suggested by Lexer He believed that fatty tissue pla ty is not surpassed by any other procedure in brain surgery *His collected material on joint plasty utilizing adipose tissue grafts comprised 165 cases with good results in 128 cases*

Koennecke (103) (1910) believed that in every skull shot wound with injury to the brain and dura a soft tissue graft—fat-fascia—is demanded to prevent rigid fixation of the brain to the skullcap The dural scar must be excised and the brain freed of all adhesions to the dura and skull In application of fat-fascia transplants the fascia remains fibrous as shown by test excision in humans and maintains a firm covering layer Furthermore the fat retains its important properties

After removal of a cyst in a head wound associated with convulsive attacks Martin (104) (1910) transplanted a free implant of subcutaneous adipose tissue from the thigh into the cyst cavity The skull defect was bridged with a free tran plant of osseous tissue from an adjacent part of the skull bone At a second operation because of continued severe attacks, the piece of fat under the bone was removed Beneath the fat the brain appeared cicatricial and necrotic *The brain began to pulsate again only after the fat implant had been removed* The cavity was covered with a skin flap and thereafter the patient s condition improved.

Macroscopically the fat was partly necrotic Microscopically retained fatty tissue rests had good nuclear taining at the margin of the section The nuclei of the intermediate tissue were increased In fat cells there was large cyst like formation with distinct stroma between the fat lobules Thick isolated connective tissue fibers lay in a large focus of a yellow crumb-like mass and this contained many well stained round lymphocytes *

Martin held that in the fifty nine days the fat tissue had been largely destroyed and replaced by cicatricial tissue although in some areas apparently normal adipose cells were observed

In four other cases of brain or dural defect associated with neurological symptoms either free fat fascia-fat or bone-fascia-fat transplants were used. Even though these cases did not speak favorably for the method with only one very doubtful successful result Martin was not induced to declare the transplantation of fat as unsuitable for replacement of dural defect and filling out brain defects

Key (105) presented two patients at the Ophthalmologic Section of the New York Academy on January 20 1910 in whom the eye had been removed and fat implants from the thigh used to fill Tenon s capsule Becoming attached to the transitional conjunctiva, the muscles make their traction from the side of the fatty stump which projects between them instead of from its summit This procedure in Key's opinion provides a better site for an artifical eye and allows more normal movement when the artificial eye is in place

Stromeyer (106) (1910) of Jena reported a case of an abscess of the lung after a wound leaving a large cavity into which several bronchial fistulas opened Two pedicled fat flaps were formed to plug the cavity The bleeding and coughing soon ceased and after fourteen days the patient had no symptoms and the wound had healed The cosmetic effect was good and the transplant fitted in completely Eight months after operation a change to connective tissue and cicatricial shrinkage were not established

Stromeyer held that free or pedicled transplanted fat surpasses every other material for closure of lung and other cavities He believed that if fat is handled suitably it has the great advantage of not shrinking and if it is not subjected to pressure or pull it will not change into connective tissue

The good results obtained by Chaput in treating ankyloses and those of Mauclaire in treating adhesions of muscle to bone established the use of the fat graft as a gliding organ A

* This is the sixth microscopic report in the literature

patient, six years of age had suppurative myositis of undetermined cause which was incised in 1912. As a result the quadriceps adhered to the anterior surface of the femur, producing marked functional difficulty In June 1919 a gluteal fat autograft was placed by Mauclaire (107) between the cut muscle and the osseous surface. Another fat graft was inserted between the anterior surface of the muscle and the skin. At the time of reporting the knee bent to a right angle

In an unpublished case a cubital nerve which was compressed by abundant fibrous tissue was disengaged at operation by Mauclaire and a fat graft taken from the thigh was placed around the nerve. This was followed by rapid amelioration of pain. There was persistent anesthesia (107)

In a plastic operation on a foot joint not previously described Reich (108) (1919) chiseled through the adhered joint mobilized the body of the joint and formed a wide space for articulation and the addition of a fat flap *

Dubreuilh (109) (1919) pointed out the mediocre vitality of the fat graft, since the least infection caused elimination of the transplant. It is very possible he stated that fatty tissue loses all or part of its fat and persists as loose connective tissue. The hard depressed and adherent cicatrix becomes supple mobile and generally level with the adjacent parts because of this loose connective tissue which elevates the depressed scar Dubreuilh considered autogenous fat transplants satisfactory for correcting old cicatrices and obtained favorable results in five patients, implanting pieces of fat from the thigh which varied in size from a hazel-nut or olive to the bulk of a little finger

Autotransplantation of fatty tissue in a skull and brain defect after shot wound gave Marchand (110) an opportunity to examine the histological behavior of transplanted fatty tissue† in the human after ten weeks healing

Microscopically a large part of the implanted fatty tissue died off in this period and was mostly destroyed and disintegrated Hollow spaces formed and were filled with fluid fat while larger portions of fatty tissue still retained their structure. Within these portions there was wide new formation of large vacuolar cells in all stages of transition from small spindle-shaped cells to large multinuclear cell bodies, which filled out the empty fat vesicles Furthermore the same cells existed in the interstitial and larger hollow spaces. These findings were traced to a proliferation of the original fat cells. Signs of immigration of small mononuclear cells (lymphocytes) were not present in these portions. It seemed noteworthy to Marchand that manifestations of proliferation were most prominent at the periphery of the lobules where the fatty tissue bordered on the connective tissue In a part of the section there were blood-filled vessels with accumulations of small round cells in the neighborhood

Concerning regenerative proliferation of original fat cells Marchand believed that two kinds of cells participate in the process proliferating fat cells and round cells in the nature of lymphocytes which can become enlarged by taking up fat These can be so similar to the proliferated fat cells that distinction may be difficult even impossible

General clinical observations made after transplantation of a fat graft were described by Julliard (111) (1920) Slight temporary swelling subsides after one or two days and only a local reaction of tissues ensues After one or two weeks the fat graft diminishes in volume and hardens somewhat. Fibroadipose tissue recovers with great ease and is maintained for a long time.

In one patient a year after transplantation of an autogenous adipose graft around a nerve suture, another surgical intervention was necessary At this time it was noted that the graft had notably diminished in volume but the fat could be identified by its yellow color and its perfectly healthy and live appearance The graft was furrowed by numerous fibrous tracts.

In a patient with an adipose graft in the region of the fossa canina, the volume of the graft appeared not to have changed for several months Evidently part of the graft had been converted into connective tissue In another patient at the end of a year the diminished perineuritic adipose graft was found to be grooved by numerous fibrous tracts but it still presented a yellow color

* The term fat flap is sometimes used by German authors to denote both free fat graft as well as a transplant with attached pedicle

† This is the seventh reference found in the literature in which an autogenous human fat graft was removed and examined microscopically

Experimentally it was established that the adipose tissue can be and is regenerated after several months the graft being maintained A large number of grafts were followed during periods varying between two weeks and six months. To quote Julliard one can conclude until proof to the contrary that the fibroadipose graft is useful clinically and constitutes an efficacious means of reparation of war and other wounds."

Heinemann (112) (1920) of Berlin held that successful plugging of suppurative osseous cavities gives an ideal result. In about 14 days the wound is healed the body parts becoming normal and the skin scar being movable in the lower layer He believed that the procedure was applicable in all locations where the skin lies against bone The only cause of failure is faulty asepsis of the cavities, since fat heals in easily everywhere in the body

In certain patients with free fat transplantation Hammesfahr (113) (1920) found it necessary to make the incision through which the transplant was to be introduced far from the place to be padded. To correct old war wounds of the face in various patients and in one with progressive hemiatrophia facialis he tried a method of blunt tunneling through which he drew the transplant to the involved region and brought it out to the periphery of the prepared bed

Douglas (114) (1920) presented to the New York Surgical Society a woman who had sustained injury to the thumb Six weeks after operation there was no flexion of the terminal phalanx. At a second operation the tendon appeared firmly united but was very adherent to the phalanx. A thin strip of fat removed from the thigh was wound around the tendon between it and the bone No adhesions reformed and function became almost perfect.

Speaking of the use of omental grafts to prevent juxtaposition Rosser (115) (1920) stated The omentum lends itself kindly and conveniently to the purpose by virtue of its neighbor like relation its pliable construction and great vascularity He found omentum useful as a placement behind and about the uterus and broad ligament In several instances free fat grafts were attached as a loosely fitting cuff in the protection of previously adherent intestines In one instance fat from the abdominal wall was transplanted between the undersurface of the liver and the duodenal portion of the stomach In a patient who had had gangrenous gall bladder perforated by stones an inlay of omentum was placed between the undersurface of the liver and the duodenum for intimate adhesions Later the fat was evidently absorbed a filmy connective tissue layer remaining only on the peritoneal side Fibrous tissue containing fat cells bound the edge of the liver to the stomach.

In a woman with complete immobility of the lower maxilla associated with cicatricial infiltration of the deep portion of the temporal muscle Juvara (116) (1921) filled the cavity after excision of the cicatrix, with a fat graft taken from the thigh. The temporal region acquired a normal aspect and movements of the maxilla became normal

Free autogenous fat grafts from the thigh were employed by Roy (117) (1921) of Montreal to fill out deep cicatricial defects from wounds of the submalar and orbital region and the cheek. The results varied from complete to satisfactory repair

Pennisi (118) (1921) preferred autogenous grafts from the anteroexternal surface of the thigh or from the abdominal wall if the strips are of moderate size but from the gluteal region or the abdominal wall for larger grafts. Following successful transfer of autogenous fatty tissue for cosmetic purpose where bony substance of the face was lost he gained confidence in the routine use of fat grafts.

From a series of experiments at the laboratories of the Division of Experimental Surgery and Pathology at the Mayo Clinic Mann (119) (1921) concluded that fat has a limited application in surgery of the peritoneal cavity the greatest benefit being in stopping hemorrhage from a parenchymal organ. In experiments with subcutaneous fat Mann found that it was not safe for routine use in patching an opening in the intestinal tract (peritoneum is better for this purpose) In the peritoneal cavity however there are possibilities for wide use of fat provided care is exercised so that it does not furnish a basis for future intestinal obstruction.

Van Hook (120) (1922) transplanted adipose tissue taken from the thigh or other convenient region beneath a pedicled skin flap to increase its thickness The fat healed in it new bed and was lifted with the skin flap when it was raised and rotated into the final implantation site

Isike (121) (1922) of the Surgical Clinic at

Bonn had occasion to examine three patients in whom Makkas ten years previously had implanted three fat grafts in aseptic and infectious bone cavities. In the first and second patients who had tuberculous osseous foci complete replacement of the implanted fat by bone had not occurred (as had been supposed on the basis of animal experiments by investigators) On the other hand in the third patient, who had had chronic osteomyelitis, there was no evidence of an osseous defect (new bone had completely filled the cavity)

Besides these three patients, twenty two others have been treated at Garré's clinic with free autogenous fat partly with and partly without success. Iseke analyzed these cases in reference to the outcome In summary he stated that replacement of implanted fatty tissue by bone does not occur in the course of healing without reaction. Slight inflammatory manifestations bring about new bone formation.

Neuhof (122) (1923) summarized his viewpoint regarding the fate of fat grafts based on the available experimental and clinical evidence as follows It may be said that transplanted autogenous fat undergoes practically the same changes as transplanted bone *The autogenous fat transplant dies and is replaced either by fibrous tissue or by newly formed fat,* the latter formed by the activity of large wandering histiocyte-like cells which take on fat and become fat cells.

Neuhof also held that the changes which occur in fat homotransplants (isogenous transplants) closely resemble those that have been described in fat autotransplants the cells in the fat graft die and are replaced either by fibrous tissue or by newly formed fat. The degenerative phenomena in fat homotransplants, however are more marked and rapid than in autotransplants. Homotransplants are not infrequently expelled even in the absence of infection.

Halstead and Caylor (123) (1924) reported on the use of free fascia and fat transplants to repair dural and brain defects

In 1925 Lexer (124) writing on research of transplantation of tissues in surgery over a period of 20 years, condemned the use of multiple small grafts of adipose tissue which do not survive as well as does a single fat segment. They tend to be overgrown with connective tissue Large proliferating cells fill the fat spaces and regress. Under favorable conditions the well nourished external layer is maintained while another part becomes slowly surrounded by cell regeneration. In the center of the graft which is farthest from the source of nourishment fat cysts are often present for a long time these become encapsulated and can be innocuous In homoplasty the power to regenerate cannot be expected in the same measure as in autoplasty with its stronger stimulation.

Lexer* contended that a decrease of about one-third in volume of the fat graft should be taken into account it does not grow in proportion to the individual He referred to a case of facial hemiatrophy in which the padding had been maintained for ten to fifteen years Application of a lipoma removed from a woman raised a depressed cheek, caused by a shot wound for four years. Transplants of fatty tissue were used in 330 large joints for support or mobility In 61 instances of freeing the adhered brain, 34 were observed longer than three years

## SUMMARY COMMENT ON LITERATURE THROUGH 1925

Free autogenous fat grafts were first used by Neuber (1893) to fill cavities resulting from loss of bony structure in the face and by 1925 surgeons had employed the transplants for a wide variety of deformities. The implantation sites of the grafts varied from clean vascular beds such as the breast, face and orbit to infected spaces in bone and lung

When free fat grafts were transplanted to uninfected and well nourished sites many surgeons believed that the transplants were clinically satisfactory This belief was based on the fact that depressions filled with free autogenous adipose grafts appeared to partly retain their elevated contour following operation. Palpation at the transplantation site demonstrated a soft structure beneath the skin which resembled the sensation produced when subcutaneous fat was palpated elsewhere in the body

The earlier clinicians disagreed regarding the amount of late absorption occurring in fat grafts and the relationship between the bulk of

* In 1919 Lexer believed that only a small part of his fat grafts was absorbed In 1925 after observing numerous patients at long intervals after operation he had changed his viewpoint and stated that about two-thirds of the substance of free autogenous fat grafts is absorbed

the graft and its subsequent reduction in size Neuber for example reported failures when large adipose grafts were used and concluded that pieces of fat which exceed the size of a bean or almond do not heal in "the smaller the pieces the more certain is a satisfactory result Later surgeons however noted greater absorption in small grafts and in multiple small segments of fat than in larger transplants. Lexer who utilized large fat grafts more extensively than any other surgeon, believed, at first that his transplants retained their original bulk. By 1925 however he reported late partial absorption in his large transplants in general about two-thirds of the grafts were absorbed

Thus by 1925 clinicians were utilizing autogenous fat grafts extensively for a wide variety of conditions, taking care to overcorrect the deformity The procedure was considered satisfactory for facial depressions deficient breast tissue filling out an orbital cavity for enveloping sutured nerves and tendons and prevention of adhesions between brain tissue and scar in patients with epileptiform seizures. Fat grafts were also widely used like bits of muscle to control bleeding Lexer reported on 165 cases in which he utilized fat grafts to restore motion in ankylosed joints, with good results in 128 cases.

Surgeons in general were transplanting larger fat grafts than seemed necessary to fill depressions so that later absorption would about equal the amount of overcorrection. Many agreed that multiple small fat grafts underwent more absorption than single larger grafts. Few however were willing to advise the transplantation of very large fat grafts excepting Lexer who used them with clinical success.

Peritoneum alone or peritoneum with a small amount of underlying fat was evaluated as a better covering to prevent intestinal adhesions or close perforations than a free fat graft The advisability of using fat grafts on brain tissue for infected sinus tracts in the lung and draining bony cavities, was open to question in the opinion of some surgeons while others held a contrary viewpoint

Strandberg in 1916 had reported his interesting case in which a pedicled abdominal skin and fat flap was transplanted to the back of the hand. When the patient took on an increase in abdominal fat following operation the transplanted fat in the hand correspondingly increased in size Thus it appeared possible that the cells in regional fatty areas retained their physiologic behavior pattern after transplantation elsewhere in the body

## Microscopic Examination of Autogenous Fat Grafts

A number of surgeons exposed autogenous fat transplants at the time of secondary operations and usually noted that some typical adipose tissue occupied the grafted area Occasionally however the grafts looked like connective tissue with no gross evidence of fat

A total of only seven autogenous human fat transplants had been biopsied or removed and examined microscopically from the time of the first implant in 1893 through the year 1925. Usually these represented grafts which were unsatisfactory or were available at autopsy and all were removed by different surgeons

A study of these seven separate reports indicated that some fat grafts were largely replaced by connective tissue (two out of seven) In the other five transplants removed at intervals from 12 days to 4 months the picture resembled that seen in animal experiments. For the first few months the histologic picture is dominated by a breakdown of fat cells resulting in the formation of cysts but in some areas of the graft apparently normal fat cells are present The graft becomes infiltrated by host cells such as lymphocytes giant cells, and polymorphonuclear cells as well as by large histocyte-like cells characterized by the German writers as *Wucherungszelle* These histocytes in the opinion of most of these investigators, took on fat and became the new fat cells in the graft, which was reduced in bulk and surrounded by a connective tissue capsule

The ultimate fat of a fat graft is dependent to some extent on the area to which it is grafted and the function it is called upon to perform The fat may be replaced by fibrous tissue or the end-result may be a regeneration of fat owing to host cells which take on fat and become fat cells The original fat cells in the graft are probably destroyed † Lexer and others on the

This statement is based on the author's review of the literature It is probable that a few observations were made which were not included in our review

† According to Neuhof the change that occur

basis of clinical experience however, believed that possibly some elements in the grafts survived

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in homotransplants closely resemble those in autotransplants but degenerative changes are more marked and regeneration of fat is a much slower process This statement regarding fresh homogenou human grafts was probably based on animal experimental work since we could find no report in the literature regarding microscopic examination of homogenous fat grafts in humans either by Neuhof or any other investigator (123)

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## II LATER TRANSPLANTATION OF FAT IN MAN

The literature on free fat transplants from 1925 up to the present time reflects much of the previous conflicting opinion regarding adipose cell survival or host tissue replacement of all fat cells in free fat grafts.

Despite the availability of antibiotics to prevent infection *free fat grafts have been somewhat neglected in recent years* No living surgeon can report operative experiences even approximating those of Lexer and other surgeons in a past era There are however trends in surgery like styles in clothes the fact that there is no satisfactory substitute for fat grafts in certain locations such as the breast and cheek indicates that with improved methods of management adipose transplants will again become popular

### REVIEW OF LITERATURE 1925–1958

Von Gaza (1) (1926) described a method of transplanting a free fat graft from the abdominal wall in a pocket in the posterior wall of the pharynx. The object of the procedure was to create a bulge in the posterior pharyngeal wall so that a patient with a short palate after cleft palate operation would have better speech. The autogenous grafts, necessarily small in children must include part of the cutis or the fascia lying beneath the fat along with the fat or a double and wider graft must be introduced.

As indications for the method von Gaza considered the following conditions when poor speech is unchanged after closure of the hard and soft palates, when only closure of the hard but not that of the soft palate is successful and when the hard and soft palate show weak development and the pharyngeal cavity is very wide

Von Gaza's experience extended to five cases of fat plasty in the retropharyngeal space Excellent speech was obtained in four patients.

Harter (2) (1927) cited a case in which fat fascia removed from the abdominal wall was inserted and anchored by suture in a depression resulting from a double Killian frontal sinus operation. After healing most of the fat had gravitated to the lowest portion of the defect. Harter found no similar case in the literature Although the cosmetic effect was far from perfect the repulsive appearance of the depressed forehead was overcome He was of the opinion that the fat graft is eventually transformed into fibrous cicatricial tissue with the disappearance of fat cells.

A subcutaneous free fat graft from the abdominal wall was used by Wrede (3) (1927) to replace half of the involved breast from which a fist-sized cyst had been removed The result six years later was satisfactory In other patients with mastopathia chronica fibrosa cystica, excellent results were obtained by transplantation of fatty tissue from subcutaneous tissue of the upper leg The cases were followed up for five and one-half and seven years respectively

Hartwell (4) (1930) believed that fat is the place where the healing process occurs and it is therefore advantageous in the healing of surgical wounds. The fat cells are replaced by infiltrating lymphocytes which grow and become macrophages. These cells take on a swollen and foamy appearance as the fat cells diminish in size Hartwell concluded that growth of the infiltrating lymphocytes is due directly to the ingestion of the fat in which they lie In wounds in humans the picture is one of replacement of fat. Fat is the site and the basis of the production of new fibrous material. The subcutaneous fat serves as the chemical basis for healing fibers the new fibrous tissue being situated along the fibrous trabeculae which course through the fat. In old long-healed wounds evidence of healing fibrosis may not be found in the fat. Hartwell noted that the histologic picture of fibrous healing in humans is different from that in animals

Moszkowicz (5) (1930) laid strips of fatty tissue taken with fascia lata from the thigh in niches in the cheek for hemiatrophy of the face. The surgical approach was through an incision in front of the auricle. There was no reaction. The cosmetic improvement was satisfactory but the late result was not seen, as the patient left for distant parts.

Subcutaneous adipose tissue and fascia lata taken from the thigh as reported by Figi (6) were inserted into the upper part of the *frontal* region just above the hairline The fat transplant filled the depression in the bone snugly and bulged the overlying skin rather tensely Six months later sufficient absorption of the transplant had taken place so that the bulging was eradicated almost entirely Since then very little shrinkage has occurred The cosmetic effect was

highly satisfactory thirteen months postoperatively. Figi held that the fat transplant may completely disappear spontaneously even in the absence of infection. The inclusion of the deep layers of the skin and the underlying fascia in a fat transplant appeared to him greatly to lessen its tendency to become absorbed. The probable explanation of this is that the increased amount of fibrous tissue present in such a graft remains even though the fat is absorbed.

Eitner (7) repaired facial atrophy in a young woman who originally had had thermocaustic treatment for hemangioma. Five years previously the scar had been excised and the right mandibular part strengthened by a paraffin prosthesis. Three years later the skin of the cheek on the left side was raised by approach through the wide scar. A fat graft from the abdominal wall was inserted beneath the skin of the cheek with overcorrection. Healing was smooth and the cheek was filled out and perfectly satisfactory some months later.

Lexer (8) (1931) reported a case of chronic cystic mastitis in which the breast was reconstructed by adipose tissue. First the glandular tissue was completely removed through the thoracomammary approach. Then fatty tissue from the axilla was rotated into the pocket under the preserved overlying skin and nipple. Lexer warned that insufficient nourishment to the transplant leads to considerable absorption and shrinkage.

In 1931 Korschelt (9) discussed the processes taking place in fatty transplants. As with other tissues the successful healing of implanted fatty tissue pieces depends on the nature of the transplant and its support on the conditions of nutrition and other circumstances. Investigations on dogs and rabbits have shown that fatty auto- and homografts from the abdomen, groin or other regions implanted under the dorsal fascia in dural defect or in different parenchymatous abdominal organs usually heal cleanly. Next a period of degeneration sets in, which is limited in autoplasty but extensive in homoplasty. Then regeneration occurs. *The same process takes place in fat transplants carried out in humans.* Cells penetrate from the neighboring host tissues especially lymphocytes, which are particularly involved in the resorption of fatty tissue. The lymphocytes take up fat and formations arise which may *be considered to be the development* of new fatty tissue structure. Under the influence of these factors there is a loosening of the finer or coarser connective tissue strands interweaving the fatty tissue. Korschelt believed that an immigration of connective tissue elements of the substratum is likely.

Although the connective tissue belonging to the transplant is destroyed under the influence of the lymphocytes and absorbed (Marchand) rests can remain behind and begin to increase and produce a small-cell tissue which participates in the structure of the whole. In general regeneration appears to come from the cell rests in the *membranes of the "fat vesicles" which underlie* the decomposition. The destruction of the main mass of fatty tissue in the transplant is accepted by Hilse and likewise the retention of strong living elements from which the reconstruction *begins. Korschelt* is inclined to believe that the* regeneration of transplanted fatty tissue occurs even under unfavorable conditions. Fatty tissue transplantation accordingly succeeds very easily and in different places. In time fatty tissue has *proved to have far reaching usefulness.*

Straatsma and Peer (10) (1932) transferred adipose tissue from the thigh to fill out postauricular depression resulting from simple mastoidectomy. The free fat grafts which were larger than the cavities required healed in cleanly giving an elevated contour. The transplants appeared to have lost about one-half of their bulk one year or more after operation and normal contour was established due to this absorption. Postauricular fistula resulting from extensive simple mastoidectomy was successfully repaired by this method in fifteen patients.

In 1932 Mauclaire (11) reviewed the uses of fat transplants as well as the uses of all other types of transplant. Grafts of fatty tissue are employed to fill dead spaces after removal of adherent depressed cicatrices and to isolate

* Korschelt states that the same process takes place in human fat grafts as occurs in animal fat grafts and cites Marchand as his authority. On the basis of examination of one unsatisfactory human fat graft buried for 10 weeks Marchand (1919) described the theoretical behavior of human adipose transplants in general. This Korschelt accepts as authoritative. Marchand was a famous man and statements by such an individual are often accepted without critical investigation by later writers.

tendons, muscles nerves, and cerebral surfaces after liberation of adherent meninges. Free implantations of fatty tissue were also made to fill cavities due to osteomyelitis and both auto- and homografts of fat were applied for the relief of flat, depressed regions.

Guleke (12) (1933) of Jena had at his disposal for study 40 cases of transplantation of large free fat grafts in the brain in which the operation had taken place more than five years previously In cure of epilepsy there was no sharp distinction between fat plastics with or without bone covering Only two out of a hundred cases since the war had a fatal outcome Clinically there was no inflammatory reaction in any instance and no effect on the neighboring brain tissue Guleke believed however that fatty tissue has a limited usefulness in substitution for brain tissue Only about forty per cent of the patients showed cure or improvement in regard to epileptiform seizures.

Guleke had an opportunity to examine fat grafts histologically after periods longer than five years, twice after eight years and once after thirteen years. The grafts were shrunken and destroyed for the most part but living residues of fatty tissue remained Where destroyed the transplanted tissue had been converted into gelatinous scar tissue the volume of which was but a small part of the volume of the original tissue mass.

Histologically the retained fat tatters showed normal fatty tissue with signs of a regenerative proliferating process as well as manifestations of degeneration the latter predominating by far

In Bennett s patient with a mastoid fistula the lining mucous membrane extending from the skin surface to the mastoid antrum was first removed and an autogenous free fat graft from the abdominal wall was introduced to fill the fistula. In anticipation of shrinkage the procedure was slightly overdone Five months post operatively the wound was closed and showed very little depression (13)

Cotton (14) (1934) presented a technique of broad undercutting insertion of finely cut fat in quantity and a modeling by hand of the area to be brought to normal level. In none of the thirteen grafts implanted by this method did a trace of reaction occur In no instance was there any extrusion of the graft material or any sinus formation Only slight temporary discoloration of the skin from blood coming to the surface was observed. *There was no later tendency to atrophy to thickening or to any fibrosis* *

Neuhof (1934) employed a fat graft from the buttock to fill a large space in the interior of the lung in a child The roentgenogram showed a homogeneous shadow and the child remained symptom-free Neuhof believed that the graft disappeared and was replaced by a more or less indifferent fibrous tissue (15)

In 1937 Neuhof (16) reported the use of autogenous fat grafts in seventeen patients with bronchopulmonary cavity or lattice lung Eleven cases showed a completely successful outcome with permanent closure Absorption and replacement by fibrous tissue can be assumed as stated by Neuhof In none of the patients in this series had the criteria for successful free tissue grafting been present namely a sterile field a bed capable of vascularization a field free from dead spaces, and one in which immobilization is feasible after transplantation. An important consideration, in his opinion, is the fact that if failure occurs after transplantation of the fat no appreciable harm is done

In 1937 Peer (17) reported the immediate replacement of a depressed frontal bone segment by a large dermal fat-fascia graft taken from the patient s thigh Two hemisections were removed from the donor site consisting of dermal fat and fascia lata so that when these were sutured together they formed a complete pattern which corresponded to the defect This permitted direct suturing of the donor site and obviated extensive deformity in the thigh. Five months after operation the patient still had a normal contour of the forehead After one year however some depression was present demonstrating that absorption had occurred in the dermal fat fascia transplant.

*Experimental work by Peer* (1937 1939) (18 19) with dermal-fat and full thickness skin-fat transplants in humans showed no macroscopic cyst formation from glands, hair follicles or from remnants of epithelium incompletely removed from the surface of the dermis. Microscopic examination revealed complete degeneration of the sebaceous glands at about three weeks

* This statement is not in accord with the experience of other surgeons using multiple autogenous fat grafts in humans from the time of Lexer up to the present

that of the hair follicles somewhat later and the ultimate degeneration of the sweat gland

*In full thickness skin-fat transplants the* epidermis degenerated and was gradually removed apparently by giant cells which were always present as a dense cellular infiltration adjacent to the buried epidermis (10)

Bremer (20) of Harvard Medical School in 1938 described techniques for staining the cell to demonstrate the existence of the thin inner protoplasmic layer of the common fat cell of adipose tissue It is an invisible film lying between the fat cell membrane and the contained fat droplet and enclosing the nucleus at one point of the circumference The cell wall consisting of this protoplasmic layer and an outer fibrillar membrane containing reticular fibers surrounds the fat droplet As pointed out by Schaffer the thickness of the outer membrane depends upon the amount of pressure that may be exerted on the cells In an individual cell the reticular membrane may be present in some portions of the circumference and absent in others

Badum (21) (1939) reported three far advanced cases of spinal adhesive arachnoiditis in which the patients were cured through operation and *free fat transplantation. Complete recovery re*sulted in the least severe case In the second case the patient with complete spastic paresis recovered so well as to be without complaint able to pursue his occupation In the third patient with tumor of the cortex of the spinal cord fat from the thigh was used to cover a large dural defect (In such a case Badum pointed out, freedom from recurrence is assured only if the cortex of the spinal cord is resected in addition to the radical operation for the tumor )

Experience with nine patients in whom autografts of adipose tissue were transferred all with good results, is reported by Urzua (22) (1939) In one patient a large piece of fatty tissue taken from the buttock was placed in depressed cicatrices of the face through an incision made in the eyebrow One year postoperatively the final result was very good but there was reduction in the size of the graft

Martius (23) (1940) utilized fat flaps from the labia majora as covering material for sutures which would not otherwise have held firmly as a result of existing lack of material A fat flap from the region of the large labia, which also contains bulbocavernous musculature was used as padding in vaginal fistula at the end of the bladder with far reaching defect of the urethra. A cavity in a fistula of the rectal sheath resulting after radiation therapy for inoperable carcinoma was packed with a pedicled fat flap from the large labia Two years later the patient was free from recurrence

Kloepfer (24) (1940) reported two severe cases of postoperative fistula, in one of which the perirectal tissue of the frontal wall of the rectum was used for covering a vesicovaginal operative fistula In the other patient a pedicled fat flap from the ischiorectal cavum covered a passage between the vagina and rectum after extensive extirpation of the uterus for carcinoma colli.

In a patient with cicatricial depression, as reported by Zeno (25) (1941) fat from the abdomen was used to correct a scar extending from the eye to the ear In a patient with submaxillary scar from chronic suppuration improvement was obtained with a free graft from the subcutaneous layer of the abdomen. Zeno applied radium systematically to each cicatrix one day after removal of the sutures to prevent the formation of excessive new scar tissue

A report by Zertuche (26) (1941) is based on *the use of fat implants from the gluteal region in* patients with enucleation of the eyeball The follow-up continued for six month to one and a half years postoperatively In two of the first patients there was considerable absorption of fat in all others the stump was very satisfactory for fitting a prosthesis.

As reported by Mezö (27) (1941) a loss of skin occurred from a burn of the palm of a two-year-old child Transference of an abdominal skin and fat flat yielded a good result Twenty three years after the operation the woman discovered that if the fatty tissue of the abdominal wall took on fat the skin fat flap transferred to the hand became thicker and thicker handicapping the patient in her activity After the fatty tissue in the region of the implant had been cut out the woman could work satisfactorily Excised fatty tissue showed no changes other than normal findings

May (28) (1941) preferred to use fatty tissue

This is similar to the Strandberg report STRANDBERG J A case of skin transplantation with unique result Hygiea—Stockholm 77: 372 1915 Cited by Well H Adipose tissue—a neglected subject J A M A 114: 2177 2184 1940

from the lateroposterior surface of the thigh, taken two-thirds larger than required to counteract degeneration and shrinkage Such autogenous fatty tissue was transplanted between the left breast and the pectoral fascia this graft showed shrinkage to two-thirds of its original size. A similar piece from the right leg with some fascia lata was transplanted into the other breast of the same patient and this showed only slight shrinkage. May's experience led him to believe that the fascia may limit the shrinkage of the graft. He further believed that the surviving fatty tissue may become atrophic but does not change character

In 1941 Neuhof (29) reported another successful case of free fat transplantation in the lung under very unfavorable conditions. The autogenous fat graft removed subcutaneously from above the iliac crest was implanted in an infected bronchopulmonary cavity Microscopic examination of a section of this graft twenty-two days later showed fat and fibrous tissue connected with granulation tissue The fatty tissue was not necrotic The wound remained healed. The specimen revealed viability Therefore Neuhof concluded *one may have to change one's viewpoint as to degeneration and disappearance of the graft.*"

As narrated by Burkhardt (30) (1941) of München, a piece of fatty tissue taken from an autotransplant showed striking changes after 16 months, namely the fat cells had died off and had yielded fat which lay as detritus beside the cells. Fat resorbing granulation tissue had developed and begun the construction of new fatty tissue

In an article describing twenty two cases of depressed cicatrices of the face corrected with adipose grafts, Urzúa (31) (1942) of Santiago de Chile reported on one failure in which the fat turned progressively into liquid and had to be extracted by puncture in the course of six months. Failure was attributed to the fact that the graft could not be nourished sufficiently since the cheek was converted into a lamina formed by skin and mucosa that had undergone sclerosis. The adipose tissue had been placed between them as inside an envelop

Bronchopleural fistulas in an open chronic empyema cavity were first closed by pedicled muscle flaps as reported by Aufses (32) (1943) A large segment of autogenous fat removed from the buttock was later inserted and the soft tissues were strapped over the fat mass. Although part of the graft became necrotic and was extruded the majority of it remained *in situ* and the empyema cavity healed completely

Fresh homogenous fat from a breast was implanted by La Roe (33) (1944) in an area of deficiency in the breast of another patient, no final report being given. A portion of homogenous breast tissue preserved for seven days was likewise implanted in the forehead of a seaman with crushed frontal bone *permanence of this "inlay' appeared to be certain after three months.* Breast tissue was also utilized to correct saddle nose with complete destruction of the nasal septum. At the time of writing the result appeared to be highly gratifying to both patient and surgeon. According to La Roe adipose tissue contains little fibrous stroma and, when transplanted under the most favorable conditions, tends to grow

Rabson (34) in 1945 presented an interesting study of the normal structure of fatty tissue. He stated that the usual conception of fat cells is erroneous in that the cell is really round or polyhedral and the nucleus is centrally placed. Blood vessels and nerves are present in fat the larger units being located in the connective tissue septa which usually subdivide the adipose tissue into lobes. The presence of vessels is important because they bring the forerunners of lipid to the cells and carry away the substances formed by the splitting of lipid. The presence of nerves is essential because they regulate the functional activity of the adipose tissue.

Basso (35) (1945) examined segments of transplanted pedunculated skin-fat flaps in humans at intervals of twelve days to about two years. Histologically there was increase in subcutaneous adipose tissue in flaps excised two to three months after transplantation The thickening of the subcutaneous tissue was of short duration and the tissue soon returned to normal.

Staining revealed cellular infiltration of fat around sweat glands and sometimes around vessels This fat infiltration began suddenly and rapidly increased until it reached its maximum two to three months after transplantation thereafter diminishing gradually until it disappeared in grafts of two years duration.

Dupertuis (36) (1946) reported the use of composite free grafts of full thickness ear lobe consisting of fat between two surfaces of skin.

A triangular wedge-shaped piece of required size is taken from the ear lobe leaving a minimal scar deformity. These free transplants are considered by Dupertuis as specially suited for use about nostrils because of shape, color and surface texture. A total of five ear lobe grafts in eleven patients were performed with success in reconstruction of the nasal tips, alar border, columella, the floor of the nostril, and small adjacent areas of deep surface scar. Dupertuis did not make observations regarding the amount of diminution occurring in the fatty layer after transplantation.

In a patient with a cranial defect of thirteen years duration from osteomyelitis of the frontal sinus, with about 2½ square centimeters of dura mater exposed, Green (37) (1947) implanted a large piece of fat with muscle sheath from the upper gluteal region. In another patient a large bony cavity in the tibia was closed with a fat graft. According to Green, fat changes readily to fibrous connective tissue and fibrous connective tissue may be transformed to bone. He pointed out that living fatty tissue may be protected from infection by use of penicillin, calcium sulfate and normal saline. These agents appear also to cause rapid transformation of the fat into solid substance.

Garelli (38) (1947) applied implantations of fat for filling cicatricial depressions from mastoidectomy and in the leveling of depressed scars following trauma or osteomyelitic processes. He also refers to good results obtained in plastic operations in respect to postoperative closing of esophagopharyngostomies with skin flaps including an abundant amount of subcutaneous fatty tissue.

Goldenberg (39) (1948) advised the use of dermal fat grafts for lifting a small depressed scar. He states that dermal fat grafts taken in one block have excellent vitality and the wound left in the donor zone is easily closed. In a few patients good clinical results were obtained by this procedure.

Autogenous adipose tissue from the thigh was transplanted by Calletti (40) (1948) in a patient with injury to the knee. A roentgenogram taken 3½ years later showed that the adipose tissue had been gradually replaced by newly formed osteoid tissue. The clear spaces in Galletti's opinion might be interpreted as vestiges of more or less modified adipose tissue.

According to Wertheimer and Shapiro (41) (1948) recent data show that adipose tissue develops from special primitive fat cells and that the cells of the tissue have a specific structure entirely distinct from the fibroblasts of connective tissue. This, as stated, tends to discredit the older viewpoint still generally taught that adipose tissue is merely ordinary connective tissue in which fat has been deposited.

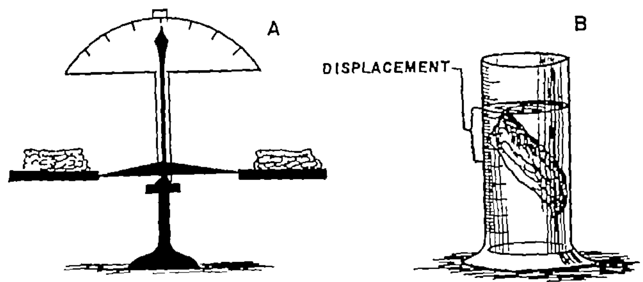
A layer of fat and fascia or a fat graft alone from the abdomen was used by Stevenson (42) (1949) to replace loss of fat in the cheek, temple and mandible. Depression of the cheek remaining after repair with a rib cartilage graft was also filled with a free fat graft. In the three cases presented the aim was to apply the grafts in about 50 per cent excess allowing for subsequent shrinkage. Good results in contour were obtained in the three patients who were examined a number of years after operation. Stevenson concluded that a large portion of the original graft will gradually disappear and for this reason free fat grafts should always be larger than the cavity appears to require.

The advantages of combining dermis and fat in small transplants were pointed out by Goldenberg (43) in 1949. He described his technique and presented illustrations of grafting depressions in the cheek. The transplants were taken from the abdomen and were well tolerated in their new location.

Cañizo Suárez (44) (1949) described a technique for radical trephination of the mastoid process and the introduction of a fat transplant in the cavity with good results. The fat is removed directly in a single mass and must be somewhat larger than the cavity so that it will remain flat or slightly convex at its outer wall, a sign that the fat is held in place under slight pressure. The advantages of the procedure as stated by Cañizo Suárez are reduction of the period of after-care (15 to 28 days), suppression of painful dressing and elimination of any disfigurement or internal or external cicatrix of the ear.

In a series of experiments on humans reported by Peer (45) in 1950 one of two equal portions of a divided segment of autogenous abdominal fat was transplanted into a pocket within the rectus sheath and the other portion cut into small pieces was similarly transplanted. A total of thirteen single and thirteen multiple autogenous fat grafts were buried in this manner and all grafts were weighed and their mass determined by the amount of normal saline which they dis-

EXPERIMENTAL FAT-DERMAL GRAFTS




FAT-DERMAL GRAFTS OF EQUAL WEIGHT AND VOLUME

E

SITES OF GRAFTS

D

SINGLE GRAFT

RECTUS MUSCLE

SHEATH

MULTIPLE GRAFTS

C

SINGLE GRAFT MULTIPLE GRAFTS

Fig 87 Transplantation within rectus sheath A. Dermal fat grafts are weighed on a sterile scale tray B Bulk of grafts is determined by immersing them in normal saline solution and recording level of fluid displacement C One of the equal sized grafts is cut into multiple small segments D The single graft and the multiple grafts are transplanted within the rectus sheath where fat is not usually present At selected intervals of time the grafts are removed reexamined for weight and volume and examined histologically The removals were accomplished during secondary operations for necessary conditions with full consent of the patients

placed. In six other instances a single segment of fat from another patient (homograft) was transplanted within the rectus sheath.*

*The autogenous fat grafts* were removed at intervals from 3 days to 14 months following transplantation. Grossly all the autografts were surrounded by a connective tissue capsule which contained fatty tissue. On the average the single autogenous grafts lost about 45 per cent of their weight and mass one year or more following transplantation. The *composite grafts made up of* multiple small segments of autogenous fat lost a much larger percentage of their weight and mass than the single autogenous fat grafts of equal size. Microscopic study supported the belief that certain durable fat cells in the autogenous fat graft receive an early and adequate circulation and these continue to survive while the remainder of the graft degenerates and is gradually eliminated. Peer believes that fat cells present in the graft one year or more after transplantation are surviving or descendent fat cells from the original graft. He did not observe host cells in the process of taking on fat and becoming fat cells.

Blood vessels containing normal white blood cells and uncoagulated red blood cells in large numbers were present as early as four days following transfer. The establishment of an early blood circulation through anastomoses of the graft and host blood vessels was therefore indicated at about four days. Areas of degeneration were scattered rather diffusely and were not specially marked in the central dependent portion of the graft. Grossly and microscopically the multiple and single fat grafts appeared like normal fat one year or more after transplantation. Microscopic examination of a section of an autogenous fat graft buried for thirteen years showed normal adipose tissue.

*The six fresh homogenous fat grafts showed* an intense early host cell infiltration, the later breakdown of all fat cells in the graft and the eventual complete replacement of the transplant by host connective tissue. *No adipose cells were seen in this host connective tissue.* The connective tissue which replaced the homogenous fat graft was so reduced in volume that it could in no way provide an acceptable substitute for the transplanted fat.

* This is the first and only reference in our records *reporting the removal and microscopic* examination of homogenous human fat graft.

From his studies Peer concluded that *free autogenous fat grafts may actually increase in size if the patient takes on an increase in adiposity which affects the particular fat system from which the graft was taken.* Fat grafts are adversely affected by trauma, exposure, infection, and excessive pressure due to a tight bandage applied over the graft.

Hoffmann (46) (1950) of the Anatomic and Anatomobiologic Institute of the University of Berlin considers the fat lobules as reticuloendothelial organs, which in the embryo have the capacity for storing fat and for blood formation. The reticulum is maintained after fat storage even if it is not visible in the cells being filled with large fat drops. But in the process of unloading the syncytial character of the tissue is again evident. The *formation of blood cells* also can again take place. Hoffmann traces the presence of undifferentiated mesenchymal cells in fat, a richly vacuolated tissue. Foci of blood formation are found not only in fat lobules but also in embryonal connective tissue, where the conditions for hematosis are favorable. Up to the present Hoffmann could not establish foci of blood formation in atrophic fatty tissue but he had only a small amount of material. If such hematosis again sets in, it comes from undifferentiated mesenchymal cells, which are found everywhere in the adult.

Fatty tissue with the capacity for fat storage undergoes differentiation, which cannot again be reversed; it maintains its specific character. The lattice fibers that are in preadipose tissue form a fine reticulum of fibers for the fat cells. They continually go over into the collagenous connective tissue sheath of the fat lobules.

Hoffmann holds that fat formation in the embryo is a differentiation process. The *anlage* of fat lobules *occurs independently of the* nutritional supply and much earlier as the panniculus adiposus forms. The lipid metabolism shows peculiarities. It is known that the fat of the infant is morphologically and chemically *different from* that of the adult. In the adult fat storage takes place not only from nutritional supply but according to the constitution."

Laro (47) (1951) corrected congenital asymmetrical face associated with congenital ear deformity by means of massive fat grafting. A tube flap constructed in the abdomen was carried by the left forearm and the end was attached to a

raw area in the left temporal region. The skin of the tube flap was peeled off and the peeled tube was buried under the lifted facial skin. The appearance of the patient was greatly improved. In a second case an alveolar sinus with depressed scar was corrected by the direct method using a free fat transplant with the fascia lata from the thigh, with no apparent shrinkage.

After a review of the literature Llanesa Rodriguez (48) (1952) reported three personal cases in which fat grafts were transplanted after radical mastoid surgery One patient in whom cholesteatoma was cured gained much hearing despite the elimination of the incus and malleus.

In three patients with progressive facial hemiatrophy as reported by Neumann (49) (1953), a double pedicled tube flap of skin and subcutaneous tissue was formed on the lower left chest and abdomen and immediately attached to the left wrist. After three to four weeks the pedicled flap was divided leaving the flap attached to its lateral pedicle and to the wrist. After another three to four weeks the lateral pedicle was divided The flap thus prepared with the epidermis shaved off was introduced into the subcutaneous space of the face with its dermis lying next to the dermis of the facial skin. Restoration of the facial symmetry was satisfactory to each of two patients.

When the flaps were revised Neumann had an opportunity for histologic study The specimens removed for study represented tissue which had been in place up to a year There was no diminution in bulk of the pedicled flaps. The pedicled flaps of dermis and fat were retained without undergoing absorption and without developing any complications such as epidermal cysts. The skin of the face was composed of epidermis and dermis and beneath this lay the dermis and fat of the flap In some regions a thin layer of subcutaneous fat of the face separated the dermis of the facial skin from the dermis of the flap There was complete disappearance of sebaceous glands with marked diminution in the number of hair follicles and sweat glands in the flap Minimal inflammatory reaction consisted of a few small collections of lymphocytes and foreign body giant cells about the microscopic remnants of hair In the fat of the flap normal adult fat cells were present

In a method of reconstructing a breast presented by Longacre (50) (1953) after subtotal or total extirpation of the mammary gland local pedicled dermal fat flaps are rotated and anchored to the pectoral fascia. He reported on four cases, of enlargement of the breast and scar tissue distortion and atrophy due to burn, in which this method of reconstruction produced a good contour

After removal of the entire diseased gland in mammectomy Malinac (51) (1953) utilized a pedicled dermal fat flap as filling material to rebuild the contour with free grafting of the nipples. He believes that retention of the pedicle prevents absorption of the fat in the graft and assures permanency of the breast contour

Barnes (52) (1953) conceived the idea that if fat grafts could be transplanted in physiologic continuity with a tissue rich in blood vessels and hence capable of prompt anastomosis, the free fat graft might survive to a much greater extent than hitherto observed. This deduction provides his present technique of lipotransplants. His first attempt was made with gluteal fat denuded of epidermis. It was estimated that there was 40 per cent reduction in size. About 90 per cent of a fat graft survived when placed with its dermal side in contact with the mammary tissue and its fascial side in contact with the pectoral fascia. Barnes recommends the use of this placement Absolute immobilization between existing breast tissue and the implant is obtained by transfixation sutures. The procedure is of value in improving contour of the breast when it is developmentally insufficient but also in recreating this same effect in the patient who has lost breast tissue through radical amputation. During the elapse of a period of slightly more than a year the results seemed to be good

After enucleation of a huge peridental cyst from the lower jaw Grandin and Deroubaix (53) (1954) filled the osseous cavity with several fat grafts taken from the patient's abdomen Six weeks after the operation roentgenographically osseous reparation had occurred. The prosthesis was being perfectly tolerated. At this time it seemed that the osseous regeneration was almost terminated.

In view of their observations they were 'obliged to admit that the bony substance which had been lost may have been reconstituted by a process preceding the graft In the majority of the cases the graft did not behave like a foreign implant

For repairing extensive defects of the face over a period of seven years Owens (54) (1955) has used a lateral neck flap usually rectangular in shape and established just anterior to the hair bearing line over the mastoid area. The compound pedicle is formed of full thickness skin, the underlying fat, the platysma muscle, the cervical fascia and down to and including the sternomastoid muscle. The advantages of using such a compound flap are that it offers a bulk of tissue and always an adequate amount of tissue for extensive facial repairs. Because of the thickness of this transplant and because of the abundant blood supply one is able later to transplant satisfactorily either bone or cartilage grafts as may be required. The compound neck pedicle fulfills all requirements of color and texture and retains its contour. Owens has found the procedure to be entirely successful.

Erdélyi (55) (1955) considers the double pedicled flap with a thick layer of fat as applicable for deep defects where not only skin but also subcutaneous tissue and fat are to be replaced. This flap consists of two large transabdominally formed flaps taken preferably from the front of the trunk and also from its lateral regions. The two lower or anterior pedicles of the flap are separated from each other by a skin bridge lying over the linea alba.

Brewer and his colleagues (56) (1955) have extended the use of pedicled pericardial fat graft to plastic and excisional operations of the trachea and bronchi in order to effect a conservation of lung tissue. During a period of five years they employed viable pedicled pericardial fat graft in 203 cases of bronchial division and closure and in 21 cases of plastic or resection operations on the trachea or bronchi. The authors stress certain technical aspects of resection for plastic operations on the trachea and bronchi, including bronchotomy, flap procedures, lobar transplants and resection and replacement of the tracheal and bronchial wall. In personal experience with 21 cases these procedures were found to be sound and were followed by satisfactory result.

A technique is described by Conway and Stark (57) (1956) for preparing migrating cross-leg and cross-thigh flaps consisting of whole thickness skin and fat as soft tissue covering for the foot and leg. The pedicled cross-leg flap is practical if the contralateral calf or thigh is sound and is of sufficient girth to supply a flap of the required size. In their series of cases 78 cross-leg or cross-thigh pedicles have been utilized in successful reconstruction in all but two cases. One to four operations were required to prepare the flaps. Conway and Stark consider these cross-leg and cross-thigh flaps to be unsurpassed in permanence, in relative ease with which they can be transplanted and in the relatively short period of hospitalization required.

In an evaluation of the clinical use of the free fat graft Peer (58) (1956) points out the necessity for a good supply of blood vessels at the host site and for the control of bleeding and oozing of blood. The fat graft is taken with a layer of dermis, unnecessary trauma being avoided and the fat graft being transferred quickly to the recipient site. Survival of the fat cells in autografts depends upon early anastomoses between host and graft blood vessels. Peer illustrates his technique for applying free fat grafts for hemiatrophy of the face. A fat graft is also indicated in underdevelopment of the face associated with absence of the external ear and in lipodystrophy.

In another article on the neglected free fat graft (1956) Peer (59) describes his method for the delayed transplantation of dermal fat graft. If the procedure is properly managed massive abdominal or gluteal fat grafts with attached dermis may "take" almost as well as moderately small adipose grafts. Donor sites for dermal fat grafts and those for delayed dermal fat graft with approximation of the defect are illustrated. Peer shows the use of dermal fat grafts in atrophy of the arm and forearm as well as for depressed areas of the face, in lipodystrophy, in congenital absence of the pectoralis muscles and underdevelopment of fatty tissue in the breast area. On the basis of his clinical experience with free dermal fat grafts he believes that the transplants are extremely valuable for the replacement of soft tissue losses. Experimental evidence indicates that about 50 per cent of the fat cells in autografts survive. All of the cells in fat homografts die following transplantation and the graft is replaced by host fibrous tissue with no fat cell replacement.

Berna (60) (1957) partly buried free fat graft following mastoidectomy to promote healing. All of his transplants were absorbed, which is not surprising since a portion of the fat was exposed in all patients.

Schorcher (61) (1957) reports on correction of

eight patients with undersized breasts among 200 mammoplastics carried out in recent years. He successfully transplanted free autogenous fatty tissue to increase the size of these deficient breasts. The mass of the fatty tissue cells and especially the connective tissue remains intact. The transplant shrinks about one-fourth of its original size which state of shrinkage it reaches in 6 to 9 months. It is insignificant whether the fat transplant is fist-sized or composed of several small pieces. Schorcher believes that several pieces are preferable from the viewpoint of nourishment of the transplant. In his oldest case of four years' duration the result is as good as those in the other cases. The transplantation of adipose tissue in his opinion has a favorable effect on the general well-being of the operated person

## CHRONOLOGIC REVIEW OF REPORTED MICROSCOPIC INVESTIGATIONS OF FREE HUMAN FAT GRAFTS

Tuffier (1911) was one of the first investigators actually to remove a fat graft and report the microscopic findings. He showed sections of a fat graft taken from the abdominal wall and introduced into the extrapleural space as an autograft at a meeting of the *Société de Chirurgie de Paris*. This free autogenous transplant four months after transfer contained fat cells which Tuffier believed to be newly formed adipose tissue rather than the original fat cells in the graft.

Zipper (1912) removed an autogenous fat graft four months after transplantation Microscopic examination showed that the center of the graft contained normal adipose cells traversed by vascular connective tissue. The transplant was surrounded by a thick fibrous capsule.

Morestin (1914) also examined an autogenous fat graft microscopically and reported that 'not a parcel of fat from the graft remained.

An autogenous fat graft was examined by Eloesser (1915) at autopsy 12 days after transplantation. The fat cells in the graft stained well but there was considerable leukocytic infiltration

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Martin (1919) removed an autogenous fat graft, previously transplanted from the thigh to the patient's brain, to alleviate epileptiform attacks due to the fat graft. Macroscopically the graft appeared partly necrotic fifty nine days after transplantation. Microscopically there were areas containing broken-down fat cells, connective tissue proliferation, and cellular infiltration by lymphocytes. In other areas fat cells with normal-staining nuclei were observed Martin believed that the fat cells in the transplant had been largely destroyed and replaced by scar

A fat graft buried for ten weeks in brain tissue was examined by Marchand (1919) Most of the fat cells had broken down and there were many histocytes and giant cells containing fat particles.

Julliard (1920) exposed several autogenous fat grafts which had been transplanted around nerves up to one year Grossly the grafts had retained their fatty character and looked like normal adipose tissue.

On the basis of published reports dealing mostly with the behavior of fat grafts in animals Neuhof (1923) drew the following conclusions The cells in both autogenous and homogenous fat grafts die and are replaced either by fibrous tissue or by new fat cells, the latter occurring through the activity of large host histocytes which take on fat and become fat cells. *Thus, autogenous and homogenous fat grafts behave like autogenous and homogenous bone grafts in contact with bone* Both fat grafts and bone grafts are replaced by the host tissue (by creeping substitution) Fat seen in an autogenous or fresh homogenous fat graft *represents replacement fat cells and not the original fat cells in the graft at the time of transplantation.*

Guleke (1933) had occasion to examine free autogenous fat grafts transplanted to brain tissue at the time of a second operation. The adipose transplants were observed five, eight and thirteen years respectively after transplantation. Grossly the grafts were shrunken and destroyed for the most part, but living residues of fatty tissue remained Where they were destroyed the transplanted tissue had been converted into gelatinous scar tissue which corresponded in volume to much less than that of the original fat graft. Histologically the retained fat portion contained fatty tissue of normal appearance with signs of a regenerative proliferating process as well as manifestations of degeneration, the latter predominating by far

For repairing extensive defects of the face over a period of seven years Owens (54) (1955) has used a lateral neck flap usually rectangular in shape and established just anterior to the hair bearing line over the mastoid area The compound pedicle is formed of full thickness skin the underlying fat the platysma muscle the cervical fascia and down to and including the sternomastoid muscle The advantages of using such a compound flap are that it offers a bulk of tissue and always an adequate amount of tissue for extensive facial repairs Because of th thickness of this transplant and because of the abundant blood supply one is able later to transplant satisfactorily either bone or cartilage grafts as may be required The compound neck pedicle fulfills all requirements of color and texture and retains its contour Owens has found the procedure to be entirely successful

Erdélyi (55) (1955) considers the double pedicled flap with a thick layer of fat as applicable for deep defects where not only skin but also subcutaneous tissue and fat are to be replaced This flap consists of two large transabdominally formed flaps taken preferably from the front of the trunk and also from its lateral regions. The two lower or anterior pedicles of the flap are separated from each other by a skin bridge lying over the linea alba

Brewer and his colleagues (56) (1955) have extended the use of pedicled pericardial fat graft to plastic and excisional operations of the trachea and bronchi in order to effect a conservation of lung tissue During a period of five years they employed viable pedicled pericardial fat graft in 203 cases of bronchial division and closure and in 21 cases of plastic or resection operations on the trachea or bronchi The authors stress certain technical aspects of resection for plastic operations on the trachea and bronchi, including bronchotomy flap procedures lobar transplants and resection and replacement of the tracheal and bronchial wall In personal experience with 21 cases these procedures were found to be sound and were followed by satisfactory results

A technique is described by Conway and Stark (57) (1956) for preparing migrating cross-leg and cross-thigh flaps consisting of whole thickness skin and fat as soft tissue covering for the foot and leg The pedicled cross-leg flap is practical if the contralateral calf or thigh is sound and is of sufficient girth to supply a flap of the required size In their series of cases 78 cross-leg or cross-thigh pedicles have been utilized in successful reconstruction in all but two cases One to four operations were required to prepare the flap Conway and Stark consider these cross-leg and cross-thigh flaps to be unsurpassed in permanence in relative ease with which they can be transplanted and in the relatively short period of hospitalization required

In an evaluation of the clinical use of the free fat graft Peer (58) (1956) points out the necessity for a good supply of blood vessels at the host site and for the control of bleeding and oozing of blood. The fat graft is taken with a layer of dermis unnecessary trauma being avoided and the fat graft being transferred quickly to the recipient site Survival of the fat cells in autografts depends upon early anastomosis between host and graft blood vessels Peer illustrates his technique for applying free fat grafts for hemiatrophy of the face A fat graft is also indicated in underdevelopment of the face associated with absence of the external ear and in lipodystrophy

In another article on the neglected free fat graft (1956) Peer (59) describes his method for the delayed transplantation of dermal fat graft If the procedure is properly managed, massive abdominal or gluteal fat grafts with attached dermis may "take almost as well as moderately small adipose grafts Donor sites for dermal fat grafts and those for delayed dermal fat graft with approximation of the defect, are illustrated. Peer shows the use of dermal fat grafts in atrophy of the arm and forearm as well as for depressed areas of the face in lipodystrophy in congenital absence of the pectoralis muscles and under development of fatty tissue in the breast area. On the basis of his clinical experience with free dermal fat grafts he believes that the transplant are extremely valuable for the replacement of soft tissue losses Experimental evidence indicates that about 50 per cent of the fat cells in autografts survive All of the cells in fat homografts die following transplantation and the graft is replaced by host fibrous tissue with no fat cell replacement

Berini (60) (1957) partly buried free fat graft following mastoidectomy to promote healing All of his transplants were absorbed which is not surprising since a portion of the fat was exposed in all patients

Schorcher (61) (1957) report on correction of

eight patients with undersized breasts among 200 mammoplasties carried out in recent years. He successfully transplanted free autogenous fatty tissue to increase the size of these deficient breasts. The mass of the fatty tissue cells and especially the connective tissue remains intact. The transplant shrinks about one-fourth of its original size which state of shrinkage it reaches in 6 to 9 months. It is insignificant whether the fat transplant is fist-sized or composed of several small pieces. Schorcher believes that several pieces are preferable from the viewpoint of nourishment of the transplant In his oldest case of four years' duration the result is as good as those in the other cases. The transplantation of adipose tissue in his opinion, has a favorable effect on the general well-being of the operated person

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A section from an autogenous fat graft buried in an infected lung cavity for 22 days was removed by Neuhof (1941). Microscopic examination showed fatty tissue which was not necrotic. Neuhof believed that the fat showed viability and that *"One may have to change one's viewpoint as to degeneration and disappearance of the graft."*

On the basis of the microscopic examination of one autogenous fat transplant, Burkhardt (1941) concluded that the fat cells died and were partly replaced by cells in the host granulation tissue which took on fat and became fat cells.

Ba so (1945) examined segments of skin and fat which were removed from the area where a defect had been covered by a pedicled skin-fat flap.* Biopsy sections were examined from twelve days to about two years after transfer. The fixed and stained sections showed increasing cellular infiltration in the fat; this reached its maximum two to three months after transplantation and then diminished gradually until it was absent in flaps buried one year and 0 months and 2 years.

Peer (1950) buried a series of measured autogenous and fresh homogenous fat grafts in the rectus sheath. A total of 20 autogenous fat grafts were transplanted and removed at intervals of 4 days to 14 months. Six fresh homogenous grafts were removed at intervals of 4 days to 7 months.

Peer observed *early death of all cells in his fresh homogenous fat grafts and eventual complete replacement of the transplant by host connective tissue with no replacement fat cells in the scar.*

Autogenous human fat grafts partly survive transplantation as fatty tissue and on the average lose about 45 per cent of their weight and volume one year or more following transplantation.

Examination of the sections did not reveal any new fat cells in process of formation from the wandering host histiocytes or other host cell. Blood circulation in autogenous fat grafts is established through anastomosis between host and graft blood vessels about 4 days after transplantation.

Experimental work was performed by the author during 1955 to clarify some of the changes occurring in autogenous fat grafts from one to six days and later following transplantation. Eighteen *dermal fat grafts* from the abdominal wall were transfixed with sutures and reinserted into the wound with the suture ends coming out through the incision. At intervals of 24 hours a suture was removed together with its attached tissue; the dermal fat graft was fixed in formalin and the tissue later sectioned and stained (58, 59).

*This series of grafts substantiated the observations made in 1950 regarding the development of circulating blood in fat grafts four days after transplantation.* The grafts buried for 24, 48 and 72 hours all had collapsed blood vessels in both fat and dermis. The three dermal fat grafts buried for 4 days, however, contained widely dilated blood vessels filled with large numbers of red blood cells and occasional white blood cells. Hence in autogenous dermal fat grafts as in autogenous fat grafts without dermis, the earliest circulating blood in the graft appears to occur about four days after transplantation.

The sebaceous glands in the dermis underwent early degeneration and were not recognizable in grafts buried for one month; the hair follicles degenerated more slowly and the sweat glands were the most persistent in retaining their structure and cells with sharp outline. Eventually, however, the glands and hair follicles in the dermis disappeared and cyst formation did not occur. The general picture was the same as observed and reported by the author in buried dermal grafts (18) and in buried full thickness skin grafts (19) transplanted in humans.

## SUMMARY COMMENT ON LITERATURE

### Clinical Experience with and Gross Examination of Fat Grafts

The literature on free fat transplantation during the thirty-two years from 1925 to 1958 gives evidence of waning enthusiasm for clinical use of the tissue. Some of this critical attitude is sound because adipose grafts were used by earlier clinicians for the correction of pathologic conditions in environments where free fat graft

The grafts examined in 1950 and the transplants which were removed in 1955 and later comprise a series of 60 free autogenous fat and dermal fat grafts removed from volunteer patients and examined microscopically.

Only six fresh homogenous fat grafts have been removed from volunteer patients and examined microscopically and all of these were described in the 1950 article.

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* Ba so's biopsies were from a transplanted pedicled flap and not from free autogenous fat transplants. The work is included because of its general interest.

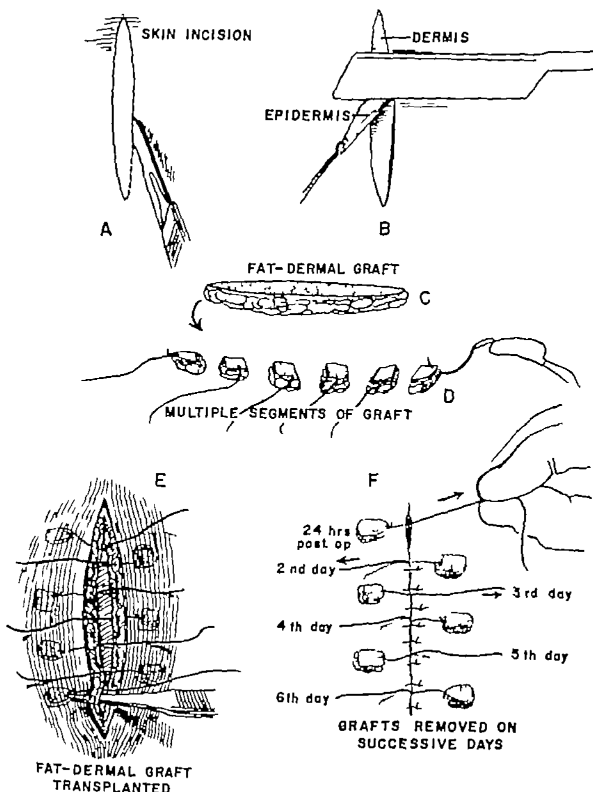



Fig 88 Subcutaneous transplantation with attached sutures A. Skin incision in abdominal region. B The epidermis with a thin layer of dermis is removed with a razor or knife C The ellipse of fat and dermis is removed by sharp dissection D Fat and dermis cut into multiple segments and a silk suture attached to the dermal layer of each segment E Dermal fat segments are reintroduced in donor site with long suture ends extending out through wound to skin surface F On successive days a suture holding wound together is removed and a dermal fat graft is withdrawn by pulling on attached silk.

cannot be successful, or in areas where successful "takes" may actually be harmful.

Free adipose grafts wrapped about sutured tendons and nerves, for example, are of questionable value because they may interfere with the early tissue fluid nourishment and delay the later blood vessel circulation in the tendons and nerves. The early postoperative swelling in and about fat grafts also causes pressure which may injure nerves. For similar reasons free fat grafts are not indicated over brain tissue or in any area where postoperative swelling may destroy delicate cells.

Fat grafts are no longer utilized to prevent the recurrence of intestinal adhesions since clinicians have found peritoneum with its covering "pavement cells" more satisfactory for this purpose. Surgeons have also given up the use of fat transplants in infected cavities and for the correction of ankylosed joints, especially in the hip, where weight bearing as well as movement are important factors.

Most of the surgeons who had occasion to expose fat grafts during a subsequent operation observed grossly that adipose tissue was present in the transplantation site. Some believed that this was a portion of the original fat graft, whereas others held that it was new fat arising from host cells which took on lipid and replaced the transplanted cell population.

Successful repairs are reported by a number of clinicians who utilized free fat or dermal fat grafts for depressions in the face and to build out deficient breasts. In spite of these reported successes, however, some surgeons appear to favor substitute hard tissue grafts such as cartilage and bone, or foreign body implants such as polyvinyl sponge.

Some plastic surgeons [Neuman (49), Longacre (50), Maliniac (51)] apparently distrustful of the known tendency to shrinkage in free dermal fat grafts, are transplanting dermal fat on pedicles with attached blood supply. The fatty layer in these transplants retains its same general bulk and the hairs and glands in the buried dermis tend to degenerate and disappear according to Neuman.

The literature contains only one reference to the successful clinical use of homogenous fat grafts (La Roe) and his patients were examined only a short time postoperatively.

## Microscopic Examination of Fat Grafts

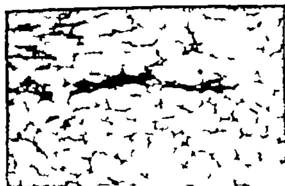
A review of the reports regarding cell behavior in free human fat and free dermal fat grafts based on biopsy study of transplants reveals a confusing and somewhat varied chain of events. Certain phenomena, however, occur with persistent regularity in free adipose grafts and these may be chronologically listed as follows.

1) From one to four days following transfer a pronounced host tissue reaction is seen about the graft and its substance is sometimes infiltrated by large numbers of host polymorphonuclear leukocytes, lymphocytes, plasma cells and a few eosinophils. White blood cells "trapped" in the graft blood vessels infiltrate the graft by diapedesis through the blood vessel walls and "trapped" red blood cells tend to become clumped and formless. The endothelial cells lining blood vessels appear viable and the fibroblasts in the stroma between fat cells also show no degenerative alterations. The fat cells have intact cell membranes and one hesitates to state that the nuclei which can be clearly seen in only a few cells are either normal or degenerating. The peripheral rim of cytoplasm does not show marked alteration when stained with hematoxylin and eosin.

The same alterations are seen in the attached dermis but host cell infiltration is less marked.

2) *At about four days the smaller graft blood* vessels become engorged with red blood cells of normal appearance and white blood cells and the latter infiltrate the graft substance through the blood vessel walls. Larger blood vessels of the graft still appear collapsed and empty. At *this stage the inference is that anastomosis has* occurred between small vessels of the graft and host blood vessels and that *circulation has become established in the adipose transplant.*

The graft blood vessels with circulating blood are lined with apparently normal endothelial cells. These seem viable even in the larger blood vessels which are collapsed or contain cellular debris. There is an increased host cell infiltration between fat lobules and individual fat cells within the graft substance. Polymorphonuclear cells, lymphocytes and an increased number of eosinophils dominate the scene with occasional giant cells of foreign body type. The small capillaries between fat cells are dilated and contain blood cells but there is no break-down of fat cells.



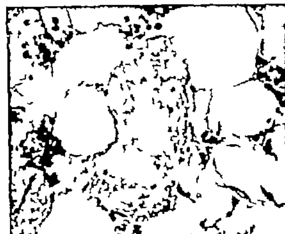


Fig 89 *Above* Autogenous human dermal fat graft buried four days on pull-out suture Note absence of cell infiltration unruptured fat cells and empty graft blood vessel ×100 *Below* The same graft showing an area with two blood vessels engorged with red blood cells and the adjacent adipose cells ×400 (Author s series)

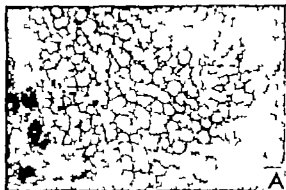
Changes in the dermis are about the same as in the underlying fat but less marked in respect to host cell infiltration Sebaceous gland cells and in some instances hair follicles show degenerative alterations while sweat gland cells appear rather normal.

3) On the fifth and sixth day the dermal fat grafts show the same general changes observed in transplants buried four days but there is more distention of graft blood vessels together with increased host cell infiltration through diapedesis (often seen) and by direct penetration of cells from the surrounding host tissue. The fat cells have intact plasma membranes and the *large typical histocytes have not yet appeared* Larger blood vessels in the graft are collapsed or filled with debris, but the endothelial cells lining these blood vessels appear viable in fixed and stained sections One notes occasional penetrating capillary sprouts from host blood vessels into the periphery of the graft. *Fibroblasts in the stroma appear viable and seem to have proliferated.*

Changes in the dermis are about the same as those described in grafts buried four days.



Fig 90 Autogenous human dermal fat graft buried within the rectus sheath 10 days Unruptured fat cells and only moderate cellular infiltration Small blood vessels contain apparently normal red blood cells ×100 (Author s series)



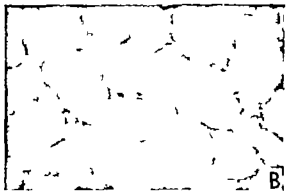




Fig 91 A Autogenous human fascia fat graft buried within the rectus sheath 12 days A rather marked cellular infiltration has appeared with numerous host histocytes ×80 B Higher magnification showing fat cells and host histocytes ×320 (Author s series)

Degenerative changes in the sebaceous gland cell however are more evident.

4) On the tenth day after burial dramatic changes are evident in dermal fat grafts *Characteristic large histocytes are present in great numbers* and the cytoplasm of these cells contains many fine particles giving them the appearance of foam cell These histocytes resemble those seen in sclerosing lipogranuloma (62) and in any area where *foreign fatty material is in process* of removal. Some of the adipose cells have broken down releasing free fat and many histocytes are present in these locations Some larger graft blood vessels are in process of becoming recanalized and contain blood cells and endothelial cell lining both of normal appearance. The fibroblasts *in the stroma between fat cells seem to have* proliferated like the endoneurial fibroblasts in free nerve grafts this indicates that they are viable The general host cell infiltration is about the same as that seen in grafts buried six days

In the dermis the sebaceous gland cells have argely broken down and the hair follicles surrounded by infiltrating host cells show marked degenerative changes The sweat glands surprisingly *appear rather normal.*

5) On the fourteenth sixteenth and twentieth days the picture is about the same excepting that there is further break-down of fat cells and increased numbers of host histocytes filled with lipid droplets. *Many unruptured fat cells however are present in the graft*

*In the* overlying dermis the sebaceous glands have completely broken down and degenerating hair follicles are often surrounded by giant cells containing hair fragments. The sweat glands are still evident but the cells show degenerative changes.

6) From thirty days through sixty days the large histocytes progressively increase in number reaching their high numerical peak in the fat graft about two months after transplantation. *In some sections the small droplets of fat coalesce in the cytoplasm of histocytes* forming several droplets or a single globule of fat. Such cells with a single globule of fat may represent tran-




Fig [illegible] Above Autogenous human fascia fat graft buried within rectus sheath 14 days Note large blood vessel general absence of cellular infiltration and unruptured fat cell X100 *Below* Higher magnification of same graft showing apparently normal fat cell X400 (Author's series)

planted and viable fat cells which are giving up fat, or those which are taking on fat *they may not all be host histiocytes* It is significant that apparently normal fat cells with intact cell membranes are always seen in untraumatized adipose grafts. Numerous small cystic spaces and occasionally large cysts are present in most sections.

In the dermis the sebaceous glands are absent, the hair follicles have largely degenerated and the sweat glands are in process of degeneration, with no evidence of cyst formation.

7) From two to eight months the characteristic histiocytes and other host cells gradually disappear and the remaining adipose cells in the transplant progressively appear quite normal. The transplanted fat contains occasional walled off cystic spaces adjacent to well formed fat cells the rather thick connective tissue capsule



Fig 93 *Above* Autogenous human fascia fat graft buried within rectus sheath 38 days Note characteristic host histiocytes and adipose cells ×400 *Below* Another area of this same graft showing a large blood vessel containing normal red and white blood cells White blood cells can be seen in process of passing through the blood vessel wall ×400 (Author s series)

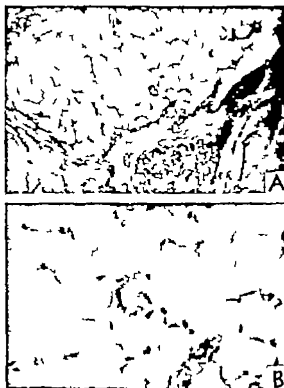



Fig 94 A Autogenous human fat graft buried within rectus sheath 60 days Note unruptured fat cells ×80 B Higher magnification of same transplant showing blood vessel containing normal blood cells and unruptured adipose cells (Author s series)

which surrounds all fat grafts, thins out considerably

The hairs and sebaceous glands are absent from the dermis and only remnants of sweat glands remain Evidence of epidermal cyst formation was not observed in our sections.

8) From ten months to thirteen years *the transplanted fat appears exactly like normal adipose tissue* excepting that it contains occasional cystic cavities and is surrounded by a thin connective tissue capsule similar to the capsules present around lipomas.

The hairs and glands are completely absent from the dermis and there is no evidence of epidermal cyst formation.

## Cell Behavior in Free Human Dermal Fat Grafts

### *Autogenous Grafts*

The two conflicting theories regarding the fate of free fat grafts are this author s hypothesis of *cell survival* as opposed to the hypothesis of *host tissue replacement*

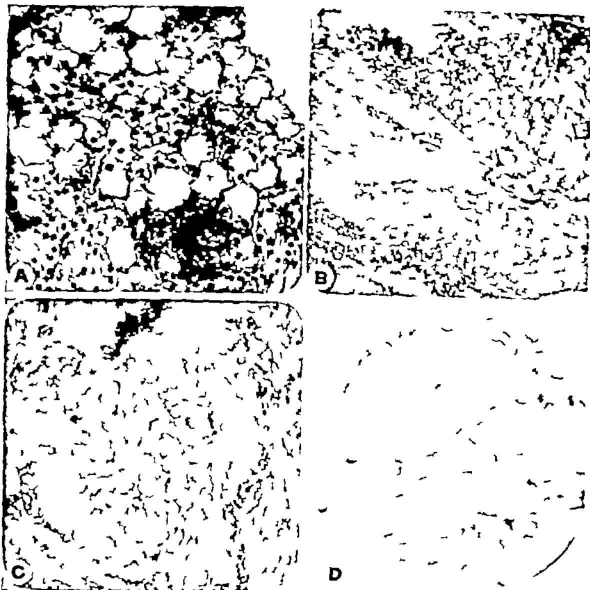



FIG 95 A Autogenous human fat graft buried in muscle for 21 days Note surviving fat cells and large histiocytes These histiocytes are absorbing fat cells which have failed to survive B Autogenous human fat graft buried in muscle 40 days Note surviving fat cells and large histiocytes which are removing broken down fat C Autogenous human fat graft buried in muscle 14 months Note normal appearing fatty tissue Graft lost about 50% of its volume D Autogenous human fat graft buried in muscle for 13 years Appears like normal fatty tissue Graft lost 50 % of its volume (Author's series)

The available evidence at this time seems to support the survival theory certain durable adipose cells in free transplants survive and it is these cells which constitute the adipose tissue eventually remaining in the transplantation site

Perhaps one of the strongest arguments against host tissue replacement is the known fact that *traumatized fat grafts are largely replaced by connective tissue* whereas gently handled fat grafts are associated with the presence of a large amount of adipose tissue in the transplantation site The host tissue replacement theory predicates that all fat cells in free transplants break down and release their fat host histiocytes then ingest this free lipid and become new fat cells If this is so one would expect that the histiocytes would take on lipid in the traumatized fat grafts and produce at least as much new fat as that which eventually remains in gently handled transplant

Apparently *factors favoring the survival of the adipose cells result in the retention of fatty tissue* whereas crushing or drying of adipose transplants brings about connective tissue replacement of the injured fat cell

The histiocytes and other host cells probably

act as scavengers to remove free lipid from those ruptured fat cells which fail to survive. When this lipid has been removed or walled off in cystic cavities, the histocytes disappear and normal adipose tissue remains, this adipose tissue represents the fat cells in the graft which have survived transplantation

The fibroblasts in the delicate stroma between fat cells survive and proliferate like the endoneurial cells in free nerve grafts.



FIG 96 *Above* Fresh homogenous human adipose graft buried 4 months beneath the rectus sheath. There are small cystic cavities and dense areas of connective tissue but no fat cells are present The bulk of the small scar nodule was 20 times smaller than that of the original fat graft *Below* Fresh homogenous human adipose graft buried within the rectus sheath cavity for 6 months Note giant cells adjacent to small cystic cavity and numerous histocytes No fat cells are present This graft weighed 21 4 gm when buried and 2 gm when removed (Author's series)

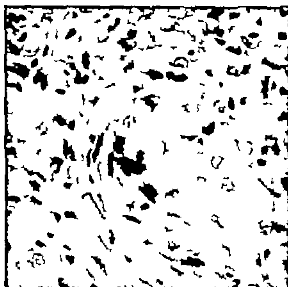


FIG 97 Hard subcutaneous nodule excised from cheek This tumor was caused by petrolatum on intranasal packing gaining entrance into the cheek tissues through the nasal cavity The petrolatum gave rise to a foreign fat reaction and numerous histocytes containing petrolatum droplets are seen in the section The picture is similar to that seen in fat homografts and in sclerosing lipogranuloma ×400

The endothelial cells lining blood vessels in the transplant also appear to survive as living cells. Circulation in the graft blood vessels occurs about four days after transplantation through anastomoses between host and graft blood vessels. The penetrating host capillaries that later invade the transplant probably anastomose with graft vessels. The pattern of vascular distribution to the fat cells however undergoes considerable change according to the chemical and physiologic requirements in different areas of the graft. Some large blood vessels do not receive circulating blood and these atrophy and disappear

The hairs and glands in the dermis of dermal fat grafts degenerate and disappear in most instances. A number of cases in which epithelial cysts occurred were described by plastic surgeons answering the questionnaire form (see Clinical Use of Fat Grafts) but these cysts were not examined microscopically Probably most of these inclusions represented fatty cysts rather than cysts lined with epithelial cells.

The fibroblasts in the dermis appear to survive in association with their collagenous and elastic fibrous matrix.

The fate of the specialized end-organs of

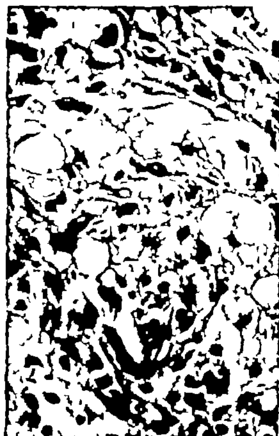


FIG 98 Section of subcutaneous fat removed from patient with sclerosing lipogranuloma following injury Note large histiocytes containing large and small droplets of lipid material which has been extruded from ruptured fat cells (Courtesy of H I Smetana and W Bernhard Arch of Path 50 296 1950)

sensation in the dermis and of the melanoblast cells has not been reported

There was evidence in the author's series that dermal fat grafts in lean individuals retained more of their original cell population and bulk than similar transplants in the obese The adipose cells in infants and young children are smaller than those in the adult.* Free fat transplants in infants and young children may retain more of their bulk (and possibly of their viable cells) that adipose transplants in adults

Free autogenous fat grafts may actually increase in bulk if the patient takes on increased fat in the abdominal donor site The fat cells in such a transplant appear enlarged due to increased lipid content

* This statement is based on a comparative study of infant and adult fat cells at the St Barnabas Rehabilitation Center

*Homogenous Grafts*

The cell population in homogenous dermal fat grafts survives only a short time and is replaced by host connective tissue cells. This host connective tissue which replaces homogenous dermal fat grafts is so reduced in bulk that it does not provide an acceptable substitute for the transplanted graft.

The microscopic picture of homogenous fat grafts shows the constant presence of *the same characteristic large histocyte cells seen in autogenous fat transplants.* These host cells take on free fat from broken down fat cells in the form of many small particles. Occasionally these small particles fuse to form a single globule in the cytoplasm of the histiocyte. The host histiocytes however *do not become fat replacement cells in homogenous fat transplants* they merely serve as phagocytes to remove the free fat Eventually when all fat has been removed the host histiocytes disappear and *no fat cells remain in the transplantation site*

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## III CLINICAL USE OF FREE DERMAL FAT GRAFTS

The transplantation of fat first attempted by Neuber (1) in humans in 1893 was widely applied by Lexer (2) but in recent years the procedure *has been neglected Although Lexer reported* numerous successful applications of autogenous fat grafts to establish normal contour in hemiatrophy of the face in small breasts and other deficiencies many plastic surgeons now tend to use cartilage bone or dermal grafts This is because they believe either that the fat grafts become infected and fail to survive or that reduction in the size of fat transplants renders their clinical value negligible

### SELECTION OF TYPE OF FAT GRAFT

#### Autogenous Grafts

In the author s experience free autogenous fat grafts with the overlying dermis provide the best available grafting material to substitute for soft tissue deficiencies in the cheek breast arm and legs In these locations dermal graft alone *do not have* sufficient bulk and must be used in a number of successive operations bone and cartilage produce hard material beneath the skin where a soft consistency is more desirable and foreign implants give rise to marble-like *structures if they are not extruded. The per*manent burial of foreign implant especially in the breast tissues may also be associated with carcinogenic change as a later result

#### Homogenous Grafts

The grafting of fatty tissue from one patient to another is a useless procedure The fat cell in fresh homografts the fibroblasts in the stroma the endothelial cell in blood vessel and all other *living elements fail to survive and the transplant* is replaced by host fibrous tissue (3) *Host tissue cells do not take on fat and replace portions of the foreign fat graft* as stated in the older literature The host connective tissue which replaces the homogenous fat graft is small in bulk and does not serve as a satisfactory substitute for the transplanted fat Thus clinical consideration of free fat grafts at this time (1959) is concerned with only one type of transplant *the autograft*

### Pedicled Dermal Fat Grafts

In selected cases it may be expedient to use fat, or fat and dermis, as a flap which carries its own blood supply instead of a free dermal fat graft (4–6) Pedicled fat grafts tend to retain their original bulk after the pedicle is severed, provided adequate circulation has been established from host tissues at the recipient site. Fat may also be undermined and shifted with a permanent pedicle from adjacent fatty areas to fill small depressions such as depressed scars the marginal skin and subcutaneous fat are then undermined rather extensively and sutured over the transplanted pedicle to provide an even contour Shallow depressions may be obliterated by shaving off the thin epidermis over the scar and covering the depressed area by undermining the marginal fatty layers and suturing them together in direct approximation *

In general the indications for undermining and shifting fat locally or transplanting dermal fat on a temporary or permanent pedicle are the same as those which govern the surgeon s judgment in shifting local or distant skin to cover epidermal losses. The simplest method is always the best method, and *free fat grafts like free skin grafts should be used largely as a procedure of necessity rather than one of choice*

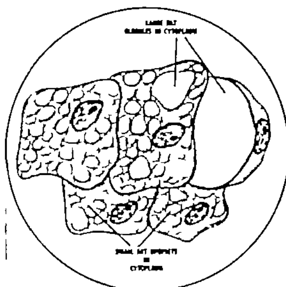


Fig 90 Typical host histocytes in autogenous and homogenous fat grafts These large host histocytes or macrophages are always seen in areas where free lipid material is present The cells which appear to act as scavengers usually ingest the free fat in the form of small particles These small fat droplets sometimes fuse to form larger droplets or a single large globule of fatty material In autogenous human grafts the histocytes appear by the tenth day following transplantation and persist until all free fatty material has been removed or is encapsulated (until about seven months)

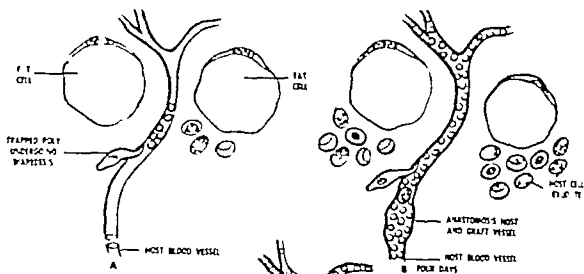
## MANAGEMENT OF FREE AUTOGENOUS FAT GRAFTS

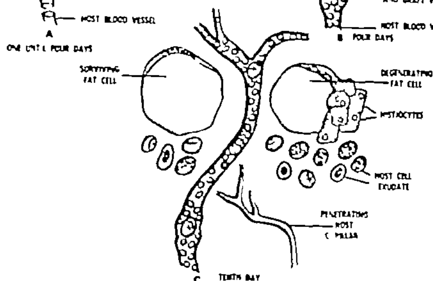
The successful management of autogenous fat grafts whether for clinical use or experimental burial is the same. The host site should be well supplied with blood vessels but all bleeding and even moderate oozing of blood must be controlled so that the bed is dry in a surgical sense. This can best be accomplished by prolonged accurate clamping of bleeding vessels and the application of adrenalin followed by thrombin. Ties with silk, catgut or other foreign materials are to be avoided except when large blood vessels are involved the ligature should include only the end of the vessel to avoid necrosis of large portions of host tissue. The dissection is made with sharp instruments or if possible, follows natural lines of cleavage to prevent trauma and subsequent break-down of tissues in the host bed

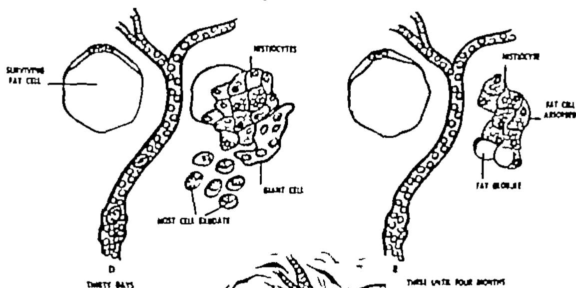
The graft is taken with a layer of dermis exposed by removing a thin split thickness skin graft with the dermatome and later using the split graft as a covering for the defect. One will create a more pleasing contour at the donor site, however if the split graft is discarded and the wound margins undermined and directly approximated The exposed dermis and the required amount of underlying fat are removed by sharp scalpel dissection, or by following cleavage lines, and the graft immediately inserted into its bed

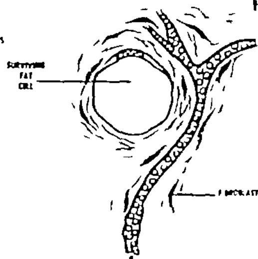
Two important considerations in removing the graft are the avoidance of unnecessary trauma and the quick transfer of the fat graft to its recipient site so that it will not lose its moist covering.

* This is a very old but useful procedure described by Celsus and Galen during the first and second centuries A.D Galen was the foremost surgeon of his day and the first experimental physiologist he demonstrated that the arteries contained blood not air as was generally taught at this time Celsus was not a physician but wrote extensively on medical subjects and in *De Re Medicina* gives a full account of skin and fat rotations which have been discovered and rediscovered many times by later surgeons.


FAT CELL
HOST BLOOD VESSEL
A
ANASTOMOSIS HOST AND GRAFT VESSEL
HOST BLOOD VESSEL
B FOUR DAYS


SURVIVING FAT CELL
DEGENERATING FAT CELL
HISTIOCYTES
HOST CELL EXUDATE
C
TENTH DAY


SURVIVING FAT CELL
HISTIOCYTES
GIANT CELL
HOST CELL EXUDATE
D
THIRTY DAYS
HISTIOCYTE
FAT CELL ABSORBED
FAT GLOBULE
E


SURVIVING FAT CELL
F

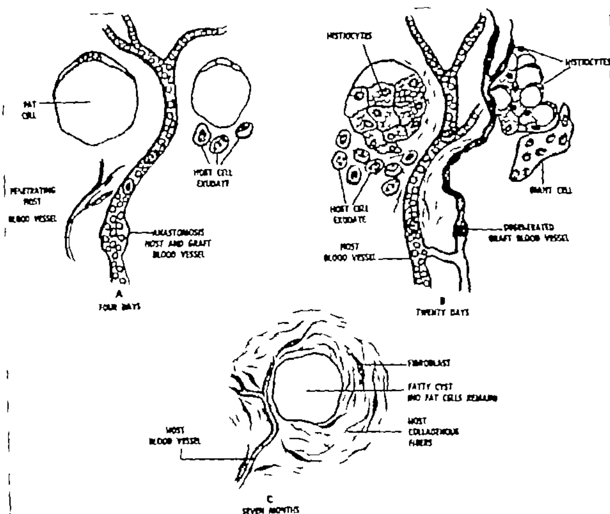



FIG 101 The fate of the adipose cells in homogenous human fat transplants The two fat cells followed in the drawings indicate the behavior of all adipose cells in homogenous fat transplants Circulation is established in the graft blood vessels at about four days as in autografts By seven to eight days however the circulation in the graft vessels stagnates and a new circulation is established through ingrowing host capillaries which supply the infiltrating host fibroblasts and host exudate cells The endothelial cells in the graft blood vessels the fibroblasts in the stroma and all other cellular components in homogenous transplants are destroyed and the graft is replaced by host fibrous tissue Drawings are based on author's series of six homogenous human fat grafts which were removed and examined microscopically

In the thigh and buttock the fat may be removed with fascia lata on its deep surface and dermis on its outer surface. One hesitates, however to remove routinely the deep fascia over the abdominal musculature because of the danger of weakening the abdominal wall.

After suture of the incision a firm but not tight supporting dressing is applied over the graft and this is left in place for one week and then reapplied for another week. Dependent drainage is recommended (48 hours)

Preoperative and postoperative antibiotic therapy is indicated to reduce the possibility of infection. If infection does occur, early dependent drainage should be instituted If infection takes place all or almost all of the graft will probably be lost With good management however this should not occur often and in

FIG 100 The contrasting fate of two adipose cells in an autogenous human fat transplant The fat cell on the right fails to survive transplantation and its fatty content is removed by host histocytes and other host cells The fat cell on the left survives transplantation and constitutes one of the apparently normal adipose cells seen in the grafted area one year or more following transplantation Drawings are based on the author s series of 60 autogenous human fat and dermal fat grafts removed and examined microscopically

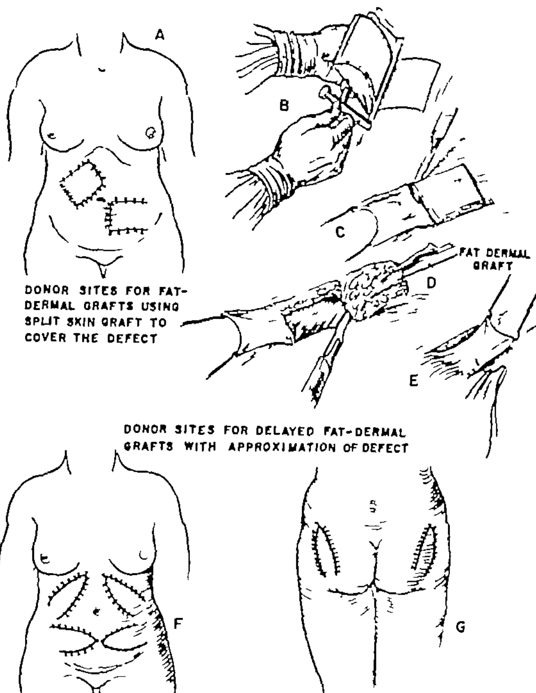



Fig. 102. A. Usual abdominal donor sites for dermal fat grafts showing split grafts sutured back to cover defect. B. Removing thin split graft with dermatome. C and D. Excising segment of dermis and fat. E. Suturing split graft to re-cover depressed donor site. F and G. Donor sites for delayed dermal fat grafts allowing two months or longer between delay and transfer. Two half sections of the transplant are removed to permit undermining and direct approximation of the wound margins. The two sections may be sutured together to form a single large transplant.

location where a soft filling substance is required one must accept this calculated risk. If the graft does become infected and is extruded another can be introduced when the area has healed. About 80 per cent of our fat grafts have been satisfactory takes.

### Size of Grafts

Moderately large fat grafts (the size of a walnut) appear to lose less bulk after transplantation than smaller multiple grafts. If the procedure is properly managed, massive abdominal or gluteal fat grafts with attached

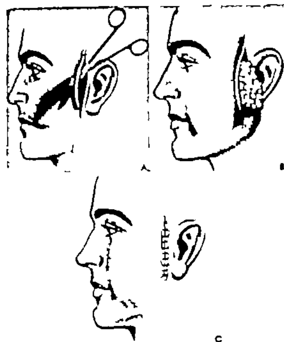



FIG 103 A An incision is made and a pocket formed in the depressed area This may extend over the horizontal ramus of the mandible and down into the neck region In some cases it may extend around the angle of the mouth and over to the mid line of the chin B Insertion of large dermal fat graft which should be sufficient in size to overcorrect the depression C Wound sutured The incision should be farther back than is indicated in this drawing as in the face lifting procedure.

dermis may "take" almost as well as moderately small adipose grafts. There is a break-down of some fat cells however in all free adipose grafts and in large transplants there will be more free lipid material than in small grafts. This free fat in combination with other materials may become encapsulated if the host cells are unable to remove it. The author has noted cystic inclusions in large fat grafts as long as ten months after transplantation In one such case where a second operation was performed to remove what was thought to be excess fat, a rather large amount of encapsulated fluid material was evacuated and normal contour was established without actually removing any fatty tissue.

Survival of the fat cells in autografts depends on early anastomosis between host and graft blood vessels, which has been observed as early as the fourth day after transplantation (3) It is therefore advisable to immobilize fat grafts for a period of two weeks to avoid injuring this newly developing circulation

The author is also using two-stage transfers of free fat grafts, allowing the transplant to remain *in situ* at the donor site for one to two months and then shifting this conditioned adipose graft to the recipient area Observations are not sufficient at this time to determine the clinical value of the two-stage procedure. Thus far none of these

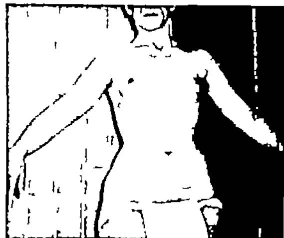


FIG 104 Patient with congenital lipodystrophy Note absence of subcutaneous fat in body surface areas above the umbilicus




FIG 105 A. Thirteen year-old child with lipodystrophy affecting all subcutaneous fat down to the level of the umbilicus Her main complaint was the depressions in the cheek regions B A dermal fat graft removed from the abdominal wall below the umbilicus has been inserted to fill depression on the left side Photograph taken one month after transplantation C Photograph demonstrating appearance twelve months after insertion of dermal fat graft in right cheek region The transplant on the patient's left has been in place sixteen months

conditioned dermal fat transplant have been lost

**Diet as a Factor in Fat Grafting**

It appears to be advantageou to put patients on a fat free reducing diet before operation. This in ures that the patient s specific fat synthesized from carbohydrates and proteins will be present in the fat cells unmodified by dietary fat Some fat cell in all free fat grafts will break down and release their lipid content and the individual s own specific fat may be less of an irritant for host tissues than modified food fat



Fig 106 Patient with hemiatrophy of the soft tissues and bone structure A large dermal fat graft ha been removed from the abdominal area and inserted to overcorrect the deformity A Photograph demon trates overcorrection one month after tran plantation B Photograph six teen month after operation demon trating large amount of absorption which ha occurred in the graft A small biopsy section removed at ten months appeared gros ly and microscopically like normal adipose tissue



Fig 107 A Large dermal fat graft transplanted to correct congenital atrophy of right side of face Photograph taken two month following transplantati n B Appearance two years after operation Absorption occurred during first eight month and remaining fat dermal ha retained it bulk since that time



Fig 108 A Child with deep depression in left cheek region resulting from x ray therapy for cavernous angioma B Appearance ten month after tran plantation of a large abdominal fat graft (without dermi ) C Appearance of this child thirteen years after fat transplantati n A biopsy section appeared grossly and microscopic ally like normal adipose ti ue

A reducing diet also serves to diminish the fat content in fat cells and this may enable the fat cells to better with tand the manipulation and surgical trauma which are unavoidable during the transplantation procedure When normal diet is resumed postoperatively the patient s abdominal fat cells take on fat and the surviving fat cells in abdominal adipose tran plant also take on increased lipid material, so that the bulk of the graft is increased. Probably about one-half of the fat cells in the grafts have failed to survive but the viable cell may compensate for this loss by increased fat storage

When the patient takes on abdominal fat the graft increases in ize when she loses abdominal fat through dieting the tran planted abdominal fat also gives up lipid and a normal facial contour i reestabli hed Free fat graft follow the behavi r of the adipose cell in th donor area and thi i evidence that the tran planted cell urvive

Clinical experience seem to in licate that fat grafts in lean individuals retain more of their original bulk than similar transplants in the obese Indeed in some cases large tran plant to the cheek region in lean patient lost so little bulk that secondary operation were necessary to remove excess adipose ti ue in the graft

## INDICATIONS FOR ADIPOSE GRAFTS

Fat i an ideal grafting material t establish normal cont our in patient with hemiatrophy

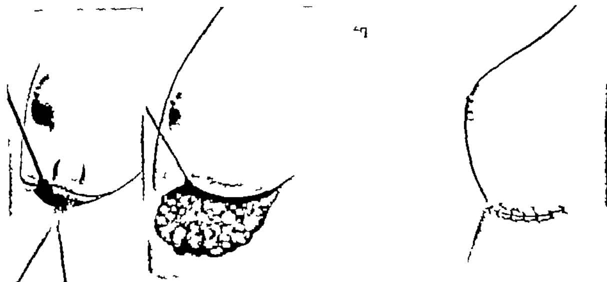


Fig 100 A An incision has been made down to the deep fascia or in congenital absence of the pectoralis muscles down to the chest cage A pocket is formed to receive the dermal fat graft B Insertion of dermal fat graft C Incision sutured

of the face, underdevelopment of the face associated with absence of the external ear and in patients with lipodystrophy

Dermal fat grafts also provide a readily available transplantation material for establishing normal contour in small breasts instead of foreign implants. Lexer and many others have used free fat grafts for this purpose and Barnes (7) reported a series of cases in which adipose transplants taken from the buttocks were utilized to establish contour in deficient breasts

Dermal fat transplants are especially indicated to fill a defect caused by the removal of a benign tumor or cyst *they should not be used after radical mastectomy for cancer* because of the danger of activating localized cancer cells and because of the fact that the skin is usually under tension and will not be elevated by an adipose transplant

Patients consulting the plastic surgeon because of small but well formed breasts must be carefully evaluated, and many should be advised to forego operation Current television programs have overemphasized standardized bust measurements and the conception of the "All American" woman with "All American" breasts is becoming a national neurosis. Female patients with extremely small breasts may have endocrine gland deficiencies and should be referred to the internist or endocrinologist before fat transplantation is undertaken. In women with small breasts and sagging skin great improvement results from successful dermal fat grafting

Patients with small lower legs and upper and lower arms due to muscle atrophy can be benefited by large dermal fat transplants when the overlying skin is sufficiently lax to permit elevation, and when there is adequate donor fat to use for this purpose.

## CONTRAINDICATIONS FOR ADIPOSE GRAFTS

An absolute contraindication to fat transplantation is *infection*. When free dermal fat transplants become infected the fat will always become liquified and the more resistant dermal portion of the transplant will usually be extruded as a necrotic mass with some attached fat fortunately the wound then heals and subsequently another dermal fat transplant may be introduced Fat grafts are therefore no longer used in infected bone cavities or draining sinus tracts of the lung.

Formerly fat grafts were employed rather extensively to fill a defect left by the removal of a brain tumor but neurosurgeons now believe that use of adipose transplants is contraindicated for this purpose the dead space is later obliterated partly by intracerebral pressure.

Fat grafts used for the repair of dural defects are not as satisfactory as those repaired with fascia transplants. Earlier surgeons employed fat grafts to prevent the recurrence of adhesions between the brain and meninges in patients with traumatic epilepsy A critical analysis of the reported results indicates failure to relieve the epileptic seizures In some patients the epileptic seizures became more severe and the fat grafts

conditioned dermal fat transplants have been lost.

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It appears to be advantageous to put patients on a fat free reducing diet before operation This insures that the patient s specific fat synthesized from carbohydrates and proteins will be present in the fat cells unmodified by dietary fat. Some fat cells in all free fat grafts will break down and release their lipid content and the individual s own specific fat may be less of an irritant for host tissues than modified food fat.



FIG 106 Patient with hemiatrophy of the soft tissues and bone structure A large dermal fat graft has been removed from the abdominal area and inserted to overcorrect the deformity A Photograph demonstrates overcorrection one month after transplantation B Photograph six teen months after operation demonstrating large amount of absorption which has occurred in the graft A small biopsy section removed at ten months appeared grossly and microscopically like normal adipose tissue



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Clinical experience seems to indicate that fat grafts in lean individuals retain more of their original bulk than similar transplants in the obese. Indeed in some cases large transplants to the cheek region in lean patients lost so little bulk that secondary operations were necessary to remove excess adipose tissue in the graft

When the patient takes on abdominal fat the graft increases in size when she loses abdominal fat through dieting the transplanted abdominal fat also gives up lipid and a normal facial contour is reestablished Free fat grafts follow the behavior of the adipose cells in the donor area and this is evidence that the transplanted cells survive

## INDICATIONS FOR ADIPOSE GRAFTS

Fat is an ideal grafting material to establish normal contour in patients with hemiatrophy

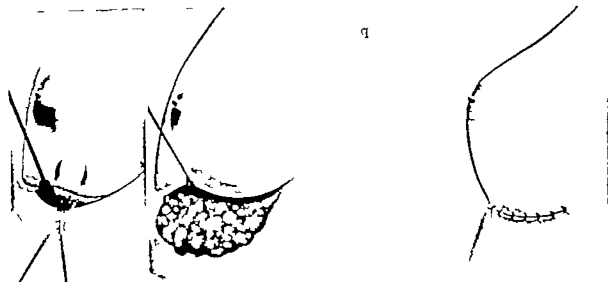


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conditioned dermal fat transplants have been lost

### Diet as a Factor in Fat Grafting

It appears to be advantageous to put patients on a fat free reducing diet before operation. This insures that the patient's specific fat synthesized from carbohydrates and proteins will be present in the fat cells unmodified by dietary fat. Some fat cells in all free fat grafts will break down and release their lipid content and the individual's



Fig 106 Patient with hemiatrophy of the soft tissues and bone structure A large dermal fat graft has been removed from the abdominal area and inserted to overcorrect the deformity A Photograph demonstrates overcorrection one month after transplantation B Photograph sixteen months after operation demonstrating large amount of absorption which has occurred in the graft A small biopsy section removed at ten months appeared grossly and microscopically like normal adipose tissue



Fig 107 A Large dermal fat graft transplanted to correct congenital atrophy of right side of face Photograph taken two months following transplantation B Appearance two years after operation Absorption occurred during first eight months and remaining fat-dermis has retained its bulk since that time

When the patient takes on abdominal fat the graft increases in size when she loses abdominal fat through dieting the transplanted abdominal fat also gives up lipid and a normal facial contour is reestablished Free fat grafts follow the behavior of the adipose cells in the donor area and this is evidence that the transplanted cells survive



Fig 108 A Child with deep depression in left cheek region resulting from x ray therapy for cavernous angioma B Appearance ten months after transplantation of a large abdominal fat graft (without dermis) C Appearance of this child thirteen years after fat transplantation A biopsy section appeared grossly and microscopically like normal adipose tissue

own specific fat may be less of an irritant for host tissues than modified food fat.

A reducing diet also serves to diminish the fat content in fat cells and this may enable the fat cells to better withstand the manipulation and surgical trauma which are unavoidable during the transplantation procedure. When normal diet is resumed postoperatively the patient's abdominal fat cells take on fat and the surviving fat cells in abdominal adipose transplants also take on increased lipid material, so that the bulk of the graft is increased. Probably about *one-half of the fat cells in the grafts have failed* to survive but the viable cells may compensate for this loss by increased fat storage.

Clinical experience seems to indicate that fat grafts in lean individuals retain more of their original bulk than similar transplants in the obese. Indeed, in some cases large transplants to the cheek region in lean patients lost so little bulk that secondary operations were necessary to remove excess adipose tissue in the grafts

## INDICATIONS FOR ADIPOSE GRAFTS

Fat is an ideal grafting material to establish normal contour in patients with hemiatrophy

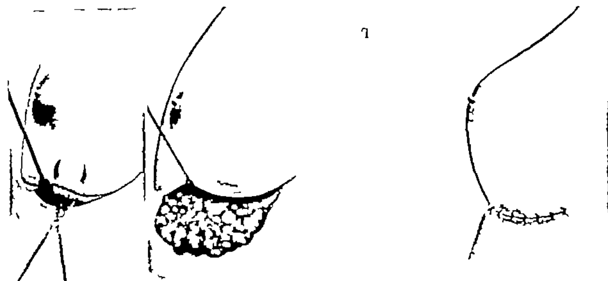


FIG 100 A. An incision has been made down to the deep fascia or in congenital absence of the pectoralis muscles down to the chest cage A pocket is formed to receive the dermal fat graft B Insertion of dermal fat graft C Incision sutured

of the face, underdevelopment of the face associated with absence of the external ear and in patients with lipodystrophy

Dermal fat grafts also provide a readily available transplantation material for establishing normal contour in small breasts instead of foreign implants. Lexer and many others have used free fat grafts for this purpose, and Barnes (7) reported a series of cases in which adipose transplants taken from the buttocks were utilized to establish contour in deficient breasts.

Dermal fat transplants are especially indicated to fill a defect caused by the removal of a benign tumor or cyst *they should not be used after radical mastectomy for cancer* because of the danger of activating localized cancer cells and because of the fact that the skin is usually under tension and will not be elevated by an adipose transplant.

Patients consulting the plastic surgeon because of small but well formed breasts must be carefully evaluated, and many should be advised to forego operation Current television programs have overemphasized standardized bust measurements, and the conception of the All American woman with All American breasts is becoming a national neurosis. Female patients with extremely small breasts may have endocrine gland deficiencies and should be referred to the internist or endocrinologist before fat transplantation is undertaken. In women with small breasts and sagging skin great improvement results from successful dermal fat grafting

Patients with small lower legs and upper and lower arms due to muscle atrophy can be benefited by large dermal fat transplants when the overlying skin is sufficiently lax to permit elevation, and when there is adequate donor fat to use for this purpose.

## CONTRAINDICATIONS FOR ADIPOSE GRAFTS

An absolute contraindication to fat transplantation is *infection* When free dermal fat transplants become infected the fat will always become liquified and the more resistant dermal portion of the transplant will usually be extruded as a necrotic mass with some attached fat fortunately the wound then heals and subsequently another dermal fat transplant may be introduced Fat grafts are therefore no longer used in infected bone cavities or draining sinus tracts of the lung

Formerly fat grafts were employed rather extensively to fill a defect left by the removal of a brain tumor but neurosurgeons now believe that use of adipose transplants is contraindicated for this purpose the dead space is later obliterated partly by intracerebral pressure.

Fat grafts used for the repair of dural defects are not as satisfactory as those repaired with fascia transplants Earlier surgeons employed fat grafts to prevent the recurrence of adhesions between the brain and meninges in patients with traumatic epilepsy A critical analysis of the reported results indicates failure to relieve the epileptic seizures. In some patients the epileptic seizures became more severe and the fat grafts

were removed with subsequent improvement in the epilepsy It seems that the postoperative swelling in and about fat grafts and the later partial break-down and prolonged host cell reaction increase pressure causing injury to delicate cortical cells

Free fat transplants have been used extensively to obviate the formation of adhesions about sutured nerves and sutured tendons. The fat, however serves as a barrier to delay blood vessel circulation for the covered nerves and tendons so the procedure is no longer used. Without a doubt early motion is the best measure to prevent the formation of adhesions about sutured tendons. If adhesions do occur a slippery fascia graft or fat shifted on a pedicle at a later operation is better than a free fat graft to provide a gliding channel for the tendons.

In the field of intra-abdominal surgery free or pedicled omental flaps are preferable to free fat transplants. The omentum contains some fat but the surface is covered by flat mesothelial pavement cells which are physiologically constituted to cover the intestines and other intra abdominal organs. Fat like cartilage, bone and tendon lives buried within other body tissues. It does not tend to survive when transplanted on a free surface.

## QUESTIONNAIRE OPINIONS REGARDING BEHAVIOR OF FAT GRAFTS

Questionnaire forms were sent to plastic surgeons requesting their opinion regarding the clinical or experimental behavior of free dermal fat grafts One hundred and eighty-seven responses provided valuable material which accurately reveals the status of opinion on the adipose transplant in the year 1958.

In summarizing the opinions names have been omitted because of the large number responding but it should be emphasized that the men who were solicited are the leading authorities in the world today

Seventy-three of the one hundred and eighty seven surgeons answering the questionnaire do not use free fat or dermal fat grafts This group consist largely of older men who did utilize fat transplants but have become discouraged by their unpredictable behavior A smaller percentage of this same group represents younger men who in following the practice of the chief of service under whom they were trained, have not employed fat or dermal fat grafts

All the surgeons using free fat grafts *prefer to transplant the adipose tissue with attached dermis* and usually select the abdominal wall, buttock or thigh as the donor sites. The donor area was closed by direct approximation following the removal of small grafts and by reapplication of the epidermis when the donor area was large. One plastic surgeon advocated delay of the recipient breast (pocket) a few days before transplantation of a dermal fat graft.

In the opinion of most of these authorities the use of dermal fat grafts was indicated for reconstruction of the breasts for hemiatrophy of the face and for lipodystrophy Underdevelopment of the face associated with congenital absence of the auricle depressions in the eyelids or cheeks and any defect resulting from loss of soft tissue were also conditions in which the use of dermal fat grafts might be indicated. One surgeon advocated the employment of dermal fat grafts in repair of hernia and a few used dermal fat or dermis alone over diced cartilage grafts to provide a smooth contour None utilized dermal fat transplants in the orbital cavity (a popular procedure in the past era) and all advised some degree of over-correction to compensate for later absorption in the fatty layer of the graft.

A number of surgeons reported the formation of epithelial cysts in some of their transplant but no microscopic examinations were made in these cases to demonstrate that the cysts were not walled off areas of lipid from broken down fat cells

Opinions regarding the behavior of the cells in free dermal fat grafts varied greatly The majority believed that most of the adipose cells died and were replaced by host connective tissue which accounted for shrinkage in the transplant. Only seven men had actually removed a section of transplanted fat and examined it microscopically these biopsies showed adipose cells with considerable connective tissue replacement or (in two cases) complete absence of adipose cell The dermis in the dermal fat grafts that were examined had undergone some reduction in size but was still present This dermis together with the host connective tissue and possibly a few fat cells constituted all that remained of the transplant. The more research-minded speculated that the remaining fat cells were probably host cell which had replaced the cell population in the transplant One pla tic

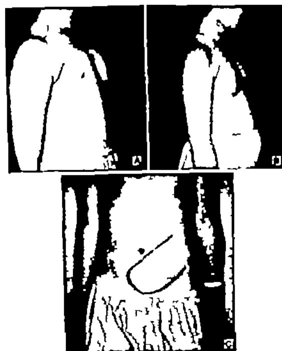
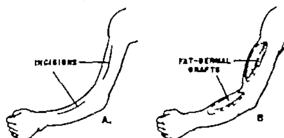


Fig 110 A Pre-adolescent child with congenital absence of pectoralis muscles and underdevelopment of fatty tissue in right breast area. B Photograph seven months after transplantation of a large conditioned dermal fat graft taken from a delayed area in the abdominal wall C Delayed area in left lower abdominal region from which dermal fat transplant was removed The donor site was incised down to the deep fascia and completely undermined two months before transplantation Theoretically it might be advisable to allow a longer interval between delay and transfer



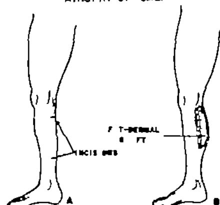




Fig 111

surgeon succinctly described the behavior of the cells in dermal fat transplants as "terrible." *

On the basis of personal clinical experience with free dermal fat grafts the author believes that the transplants are extremely valuable for the replacement of soft tissue losses. Thus far he has had no failures (loss of the transplant) with grafts in which the fat cells were conditioned or delayed one month or more before transfer Delayed dermal fat grafts also appear to retain more of their original bulk than directly transplanted adipose grafts. Whenever grafts were exposed at a later operation to remove excess tissue or add more the transplants looked grossly like adipose tissue and this was confirmed by microscopic examination. Not infrequently cysts containing broken-down lipid were present as walled off and well tolerated cavities surrounded by adipose cells of normal appearance we have encountered only one true epithelial cyst in a total of about ninty experimental and clinical grafting procedures.

The pretransfer delay of the recipient breast pocket, as suggested in one of the questionnaire responses,** is worth considering

One point not previously mentioned is whether to insert the dermal fat graft with the adipose layer beneath the skin or vice versa We have used both positions and have concluded that either method is satisfactory From a clinical standpoint there is no apparent relationship between the position of the graft and the successful take, satisfactory contour or amount of postoperative absorption

## SUMMARY, MANAGEMENT OF FREE DERMAL FAT GRAFTS

1) Routine antibiotic therapy is given before and after operation

* The late Dr Milton Adams

** In his questionnaire response Dr Benjamin F Edwards states that he has tried this method devised by Dr M K Rueh A sponge soaked in Achromycin is inserted into the delayed breast pocket three days later the sponge is removed and a dermal fat graft is inserted into the cavity

2) A preoperative routine fat free reducing diet is instituted one month before transplantation.

3) Fat and dermis are delayed one month or longer before transplantation.

4) All bleeding in the recipient site is controlled with adrenalin and thrombin, ligatures being used only for large vessels.

5) Both the graft and the recipient pocket should be subjected to a minimum of trauma.

6) After all bleeding has been controlled in the recipient site, dermal fat should be removed and the graft transplanted immediately.

7) The dependent part of the transplantation site is drained for forty-eight hours after grafting.

8) The grafted area is immobilized for at least ten days postoperatively.

9) The donor site is repaired by direct suture whenever possible instead of split graft coverage. A large graft may be obtained by removing two dermal fat segments from different areas so that the total bulk of the two transplants is sufficient to fill a particular depression. The donor sites may then be covered by undermining and direct approximation.

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# PART V

# *Nerves*

CHAPTER 8

# Transplantation of Nerves

STERLING BUNNELL

## INTRODUCTION

Much experimental work both in laboratory animals and in man has been done on nerve grafts, in various states—refrigerated, preserved by chemical, dead living—and of various kinds—heterografts, homografts, and autografts. A foundation of definite knowledge has been laid down. It is now known that all of these grafts with the one exception of autografts are unsuccessful in man. It is possible that when a means of preventing tissue immunity is found homografts may be successful.

Throughout the literature there have been many enthusiastic reports of good results with nerve grafting by methods quite impossible of success, and one must sift them out and judge tolerantly but with appropriate incredulity. Such reports should be scrutinized for documentation and details of the degree of success with insistence upon absolute proof of regeneration. Overlap and natural improvement must not be misinterpreted, and wishful thinking should not bias judgment.

In this chapter the work on hetero- homo- and autografts will be reviewed and then autografts—their history evidence of success past surgical errors, and the technique of repairing gaps in peripheral nerves—will be discussed.

## HETEROGRAFTS OF NERVES

Heterografts are taken from one species and placed in another. There has never been a published report of success with this method that bears up under careful scrutiny and there are numerous reports showing that such a graft is just an irritating foreign body does not become fused with the host, and is eventually absorbed.

Gosset and Bertrand (1) in 1937 fixed the spinal cords of cats and rabbits in formalin, then alcohol, and grafted 8 mm. to 3 cm. into the sciatic nerve of a dog. They reported that the dog used his leg and stood with his heel high. They also described fast permeation of the graft and a very good result. Gosset (2) in 1937 reported an 11-cm. graft from a rabbit, a 5-cm. one from a cat, and a 3-cm. graft from a rabbit in the radial median, and sciatic nerves in man with regeneration in 2 to 2½ months. Twelve months later the wrist extended in the case of the radial nerve, opposition returned in the case of the median nerve, and 5½ months later muscle showed electric reaction. The thumb and fingers moved incompletely. In the case of the sciatic nerve, in 20 days toes started to flex and in 3½ months the patient walked well.

Gosset (3) in 1938 reported a collection of 27 grafts of spinal cords of rabbits fixed in formalin and alcohol. He reported excellent improvement after a 5-cm. graft of cat cord in median nerve. In 3 months he obtained opposition. After a 3-cm graft in the sciatic nerve the patient walked. A case of a 5-cm. graft of rabbit cord in the median nerve in 9 months showed complete opposition and return of sensation.

Nageotte in 1937 and 1939 (4, 5) reported the graft of formalinized nerve from veal and the spinal cord of rabbits into man with good loss of anesthesia. He stated that the chemically preserved graft was better than fresh as there was much less reaction.

Björkesten (6) of Finland in 1948 reported 65 cases of heterografts by the method of Gosset

and Bertrand and stated that in no case did he obtain regeneration nor did Sjoquist in similar cases

Weiss (7) used dehydrated frozen grafts both heterografts and homografts and placed sleeves of dried frozen arteries about them. The grafts were rehydrated before planting. He reported the sleeve method to be good and claimed to get full functional recovery in cats and monkeys from the hetero- and homografts

To quote F K. Sanders (8 9) from a report in 1942 Heterografts are now only of historical *interest. There is no single instance in the* literature of nerve grafts between two mammalian species being followed by any recovery which would stand up to critical examination Only a few axons grow There is a strong tissue reaction the graft does not heal in Schwann cells do not proliferate and the graft does not undergo Wallerian degeneration. Chemically preserved heterografts are of no more use than fresh ones.

## HOMOGRAFTS OF NERVES

Homografts would fill a need in man if they were successful as the surgeon often needs to bridge a large gap and sufficient autografts are not readily available. Short homografts have been successful in rabbits and cats but never in man, and even in rabbits and cats long homo-*grafts are ineffective. The axons can be seen* growing down the graft at about the same speed as after a nerve suture, but when they reach about 2 cm. growth stops, for by that time the graft commences to die and the axons can penetrate no farther As with all other homografts, except of cornea the immunity of the host kills the nerve graft. If the transplant is only about 2 cm. long it acts as a trellis and the down-growing axons reach and penetrate into the peripheral nerve fragment and grow on down it It is a race between the growth of the axons through the graft and the development of active *immunity in the host*

Bentley and Hill (10) in 1936 compared autografts and homografts 3 cm. long in cats The axons grew faster through the autografts but the homografts showed much more foreign body reaction than the autograft becoming filled with leukocytes When the axons passed through eventually all of the graft was absorbed leaving the axon intact but the nerve enervated the muscles below in both cats and dogs A length of 3 cm. was found to be the limit for homograft the tissues being compatible with the host only at first and later being absorbed. Bentley and Hill noted connective tissue proliferation in the graft They used 20 cats and monkeys. In eight monkeys 3-inch popliteal homografts were used and in 500 days some motor response was found in the leg and growth of nerve fibers as far as the ankle was observed.

Spurling (11) in 1945 placed eight homografts in man and reported that none of them was successful. On sectioning the grafts which he *found had become fibrous, he noted that axons* had grown down them to the following lengths

| Growth of Axons mm. | Length of Graft mm. |
|---|---|
| 40 | 40 |
| 18 | 5 |
| 15 | 78 |
| 23 | 00 |
| 5 | 70 |
| 10 | 32 |
| 10 | 38 |
| 20 | 20 |

*Barnes (12) reviewed reports on autografts* by Bentley and Hill Sanders and Young (42 rabbits) in 1942, Seddon and Holmes in 1944 and Spurling Lyons, and Woodhall in 1945, *all of* whom claimed unqualified failure. Barnes further reported complete failure in eight cases of large homografts in man and accounted for it by the tissue reaction to the graft. In one case the axons reached only 25 mm. and the 18 cm. of the graft became fibrous. In another the axons reached 12 mm. and the graft became fibrotic and necrotic. Barnes noted six other cases of similar results with failure ascribed to actively acquired immunity *Sanders and Young (9)* in 1942 placed 2 cm. of homografts in 35 rabbit The grafts became surrounded by a fibrous sheath with blood supply but were swollen had a less blood supply and showed more adhesions than autografts The growth of axons was impeded by the reaction and there were more microphages and lymphocytes present

Holmes (13) in 1952 stated that the axon mu t pass through *the Schwann cell* which constitute the tubes and the Schwann cell must grow with the axons Together they make a myelin sheath Holmes charged that report of success ful homograft *did not hold up under*

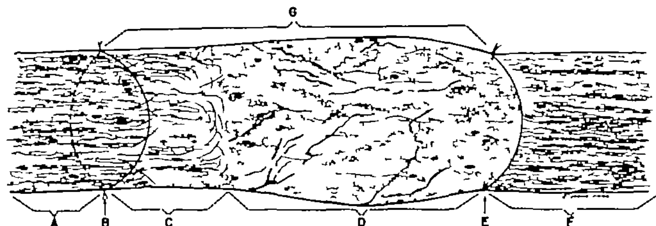



FIG 112 Schematic drawing of homograft B to E Graft Proximal segment on left C Axons growing down only 2 cm D Disintegration of homograft by active immunization of host Dilated blood vessels macrophages and round cells are seen A. Proximal stump F Distal stump

scrutiny although Duel reported three of tiny seventh nerve grafts.

Davis and Ruge (14) in 1950 said "Many investigators have studied the transplantation of nerve homografts and have discarded the procedure, maintaining that the graft produces a foreign body reaction in the host and is reduced to a fibrous band or is absorbed entirely

They transplanted fresh sciatic nerve homografts in 35 cats. Microscopic examination of sections of the grafts indicated that the superficial layers retained their viability whereas the deeper areas often were necrotic Where grafts were viable the original fascicles were unchanged and the endoneural tubes remained. Regeneration of the nerve was always spotty but adequate in the peripheral layer One year following insertion of the grafts all animals were capable of nearly complete motion of the involved foot and toes. These investigators found no difference between the junctures made with plasma and those with silk.

Homogenous nerve grafts were also performed upon two patients. The grafts were taken from recently amputated extremities or from cadavers soon after death and were refrigerated from 30 minutes to 45 hours before being used. In regard to results the authors stated, "While none of the patients have shown any motor function or enough evidence of regeneration to approach normal some have shown minimal evidence of recovery" Three of the nerve grafts were exposed and examined 4 to 8 months following transplantation, and in no instance had they been absorbed or reduced to a fibrous band. In attempting to explain the markedly inferior results in man compared to the experimental results the authors emphasized the importance of the condition of the graft itself

Sanders (15) in 1954 said in reference to homografts

Pieces of fresh nerve transplanted to fill a gap in a nerve of another individual of the same species become like autografts firmly united with the host nerve although thereafter their behavior is very different In favorable circumstances the distances reached after 15 or 25 days by nerve fibers growing through these grafts are as great as those through autografts Most grafts however show far shorter distances of outgrowth, and when treated statistically the data show evidence of a significant variability not under experimental control (Sanders and Young 1942) Recovery both of motor function and cutaneous sensibility can nevertheless follow the use of short homografts in the rabbit e.g six out of seven such grafts investigated by Gutmann and Sanders in 1942 recovered motor power of a quality little inferior to that given by autografts Four of the six animals which showed motor recovery also had some return of cutaneous sensibility

The variability in the distances reached at a given time by new fibers growing through homografts is reflected in their histological appearance Wallerian degeneration takes place more slowly in homografts than in autografts so that unbroken myelin segments can be found as late as 25 days (Fig 113) The result of this delayed break-down of the contents of the graft can be seen at 60 days when a graft may contain large spaces full of active macrophages (Fig 114) even 200 days after grafting similar though smaller macrophage-filled spaces remained (Fig 115) It is uncertain however how far these later appearances reflect the process of Wallerian degeneration (which although

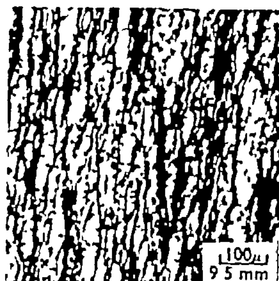



FIG. 113. Part of a 2-cm. autograft 25 days after insertion, showing resemblance to distal stump. Note Schwann bands and spaces filled with microphages. (From F. K. Sanders, Peripheral Nerve Injuries, Medical Research Council, Her Majesty's Stationery Office.)

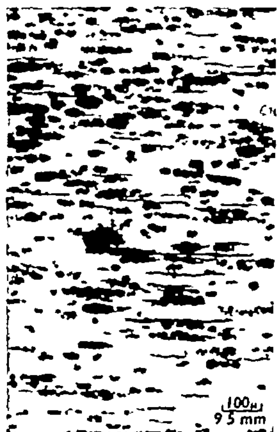



FIG. 114. Autograft after 25 days showing normal disintegration but *slow removal of myelin*. New fibers in the graft are already becoming myelinated. (From F. K. Sanders, Peripheral Nerve Injuries, Medical Research Council, Her Majesty's Stationery Office.)

flow is fairly normal in its early stages) and how far they result from a deep-seated reaction from the host to the graft which causes the latter to be destroyed and replaced partially by the host tissue.

By far the most striking feature of homograft histology is their invasion by lymphoid cells. The degree of invasion is very variable. In the best case *infiltration is only minimal* but in less favorable instances the whole graft becomes distended by a mass of lymphocytes and its internal architecture considerably disturbed (Fig. 116); eventually there are patches of necrosis in extent proportional to the intensity of this reaction. Although the lymphocytic infiltration subsides fairly quickly, there is inevitably a certain amount of fibrosis and less uniform innervation than in autograft. Although new nerve fibers are able to penetrate homografts and to become myelinated within them, parts of such grafts usually at the center contain either no or only small nerve fibers (Gutmann and Sanders 1943). However, in spite of such unfavorable features, nerve homografts in the rabbit are still able to conduct nerve fibers across a 2 cm. gap in sufficient numbers to produce a return of motor function and of sensibility only inferior to that of autografts.

The Nature of the Homograft Reaction. The apparent success of nerve homografts in animal experiments is not paralleled by any similar result in man (Sanders 1942; Seddon and Holmes 1944). Very few human nerve homografts have been followed by any recovery of function which could be unequivocally ascribed to the affected nerve, and sections of such grafts removed at long *intervals after insertion have shown them mainly* converted into fibrous strands. The reason for the discrepancy between the results of homografts in man and in experimental animals can be found in the nature of the host reaction to homograft.

In the case of skin transplants, Medawar (1944, 1945) has shown that homografts behave at first like autografts, the grafts healing securely and even giving rise to epithelial proliferation. However, after a variable period an acute inflammatory reaction sets in and the whole native cell population of the graft is destroyed. The inflammatory process includes first of all vascular and lymphatic proliferation, a mass invasion of the graft by lymphocytes and monocytes of host origin, severe edema, and a general mobilization of mesenchyme cells. At the close of this phase a stagnation and obliteration of the vascular system of the graft together with death and necrosis of its constituent *cell population takes place*. Later homografts are invaded anew by fresh vessels from the host and the lymphocytes and monocytes pass through their walls to establish a secondary cell population within the graft.

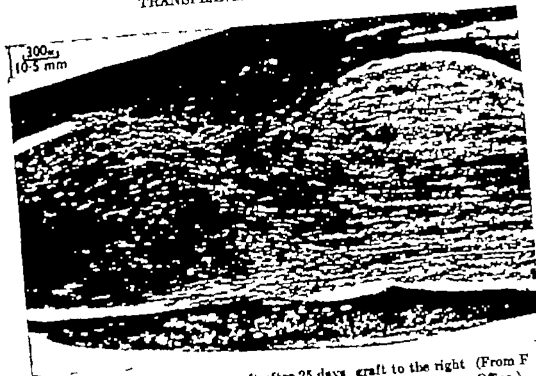



Fig 115 Proximal junction of an autograft after 25 days graft to the right (From F K Sanders Peripheral Nerve Injuries Medical Research Council Her Majesty s Stationery Office )

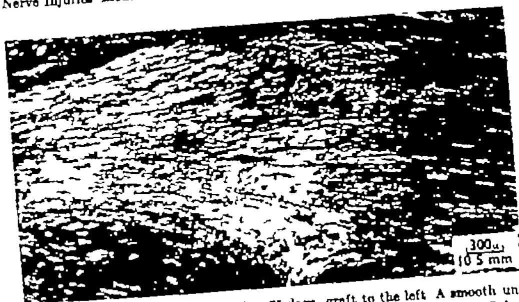



Fig 116 Distal junction of an autograft after 25 days graft to the left A smooth union has been made although no fibers have yet arrived (From F K. Sanders, Peripheral Nerve Injuries, Medical Research Council Her Majesty s Stationery Office.)

"The whole reaction of the host to the grafted foreign tissue is of the type of an actively acquired immune reaction and consequently depends qualitatively upon the amount of foreign tissue transplanted Thus a single small homograft of skin may survive many days without destruction while larger amounts of tissue—whether trans planted as a single large or many separate small grafts—undergo a much more rapid breakdown Moreover second-set grafts from a given donor to a host already immunized by grafts from that donor break down even more quickly Such ob servations are of particular interest since in the case of nerve grafts one of the main features distinguishing human and animal homograft experiments has been the size of the transplant used Most animal experimenters have used small thin grafts 2 or 3 cm long while most of the grafts used to repair human nerve defects have been 8 to 10 cm in length and of correspondingly greater diameter Such grafts represent a much greater dosage of foreign tissue than has been used in animal experiments and provided nerve homo-grafts behave similarly to skin the more rapid break-down of such large grafts might be expected adversely to affect the recovery It is interesting

to note in this connection that the only human nerve homografts to show any convincing evidence of recovery were three of Duel's 1933 six cases of facial nerve homografting. In all of these the dosage of tissue was low, the nerve transplanted was of small diameter, and the gap to be bridged was short.

Sanders also stated (15): "Histological investigation of inflammatory changes which take place within nerve homografts has shown that the response of the host tissue to such grafts resembles that seen in skin grafts (Medawar 1944–1945), although with nerves the reaction is complicated by the activity of the nerve fibers and Schwann cells of the host nerve into which the graft is inserted. In the case of a nerve homograft, primary union between the graft and the host nerve takes place. Wallerian degeneration begins within the graft together with proliferation of its Schwann cells. New blood vessels coming mainly from the ends grow into the graft and nerve fibers from the central stump cross the upper junction, start to travel down the Schwann tubes of the graft, and may begin to myelinate within it. The lower junction is made by Schwann cells from both the graft and the peripheral stump.

Parallel to these changes and with an intensity dependent upon graft dosage, inflammatory reaction builds up. Mononuclear cells pour out through the blood vessels into grafts which become edematous. The reaction is most intense at the junctions which at 25 days are markedly swollen and packed with lymphoid cells; large dilated blood vessels can be found within the graft, especially in the neighborhood of the junctions. At the height of the reaction the walls of the vessels break down and local hemorrhages occur within the graft. At this time the native cell population of the graft is destroyed. At this time, therefore, new nerve fibers and Schwann cell strands from the host nerve which have invaded the graft from its two ends are exposed to an environment in which the only structures maintaining the continuity of the nerve are the collagenous tubes of the graft. In short grafts this is without serious effect, since fiber and Schwann-cell continuity is established throughout the length of the graft by cells of host origin in the period before breakdown takes place. These structures seem relatively unaffected by the short period of ischemia which results from the breakdown of the blood vessels of the graft, since short nerve homografts removed at long intervals after insertion closely resemble autografts, having been repopulated by cells from the host nerve. The accumulation of microphages seen in such grafts in the latter stages probably have to do with the removal of the dead and necrotic remnants of the original graft contents. The fate of the graft collagen is less certain. Medawar 1945 found the collagen of skin homografts to be removed eventually and substituted by host collagen. Although this took place much later than the destruction of the cells of the graft, phagocytosis of the graft collagen fibers did not take place. The collagenous framework of nerve grafts may also be replaced by host collagen. If so, the new collagen must be laid down in a pattern of the old, since the latter undoubtedly acts as a guide for the nerve fibers and Schwann cells as they traverse the graft. This might account for the slight interstitial fibrosis seen in the late stages of successful low dosage homografts.

In long grafts the bigger dosage of homologous tissue causes the inflammatory reaction to come on earlier and to be more intense. Primary vascular breakdown and destruction of the native cell population of the graft takes place before it has been penetrated throughout by blood vessels, Schwann cells or nerve fibers. Regions thus exist in the middle of a long homograft which have been without blood supply ever since transplantation. Such a region later becomes heavily collagenized and can act as a barrier to regeneration. As a result, a long nerve graft examined at a late stage often consists of a thick fibrous strand at either end of which are segments resembling normal nerve; that at the upper end looks like a re-enervated distal stump, that at the distal end resembles a degenerated distal stump of long standing (Holmes and Young 1942). Histological appearances were found in human nerve homografts removed following the failure of regeneration. What counts most against the success of a large nerve homograft is the fibrosis of part or all of the graft. The feature of the homograft reaction which seems most damaging from this point of view is the breakdown of the blood vessels growing within the graft and not the death of the constituent cell population. It is thus possible that some treatment of homograft which would modify the homograft reaction in the direction of avoiding primary vascular breakdown might improve the prospects for homografting, whether or not it destroys the cells of the graft."

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Seddon (16) in 1944 implanted four homografts in human subjects and months later when the grafts had not regenerated removed three of them for microscopic study They had become collagenized both from without and within and so were reduced to fibrous cords devoid of any nerve bundles within them.

## UNDESIRABLE METHODS OF NERVE GRAFTING

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Tubulization has been a persistent human error through the years in making nerve junctures For this arteries and veins have been used or the nerve has been wrapped around with deep fascia or preserved animal membrane. Of late tantalum foil has been used. Tubulization is non-surgical, especially with a nerve graft as it robs the nerve of blood supply Whole tubed grafts have been seen to be necrotic throughout for this reason. Tantalum foil is especially pernicious. It was used to push scar tissue away from a nerve and to guide a smooth covering for it, but flexible foil cannot push away tissue. It was used extensively in the last World War but now its presence is an indication to remove it. It has prevented many a nerve from regenerating, although some sutured nerves have regenerated in spite of it. When it is placed in movable tissue, as opposite a joint, a mechanical conflict is set up since no metal withstands constant motion. The foil fragments into many curled particles and from irritation there is free yellowish fluid granulation tissue, and a great fibrous proliferation around the whole.

To prevent a nerve from becoming strangulated or infiltrated by cicatrix, which is a frequent cause of failure, it should be placed through good tissue either by re-routing or by placing good tissue beneath it or around it. Whenever necessary a pedicled skin graft should be placed first.

A nerve juncture in the presence of infection cannot be expected to yield as complete a regeneration as one done in a clean field. The degree of regeneration is in direct proportion to the microscopic accuracy of the union. Infection detracts from the accuracy of approximation and is productive of cicatrix. Many such junctures of the facial nerve have been made in granulation tissue and with open drainage, but in spite of this some have shown a degree of regeneration

Predegenerated grafts, first proposed by Cajal, were advised and used by Ballance and Duel on the theory that the grafts were prepared ahead of time for the reception of the axons, and the tubules would be open Those authors severed a branch of the anterior cutaneous nerve of the thigh a few weeks before and took the graft from the peripheral portion. They stated that the axons then rushed down fast, resulting in regeneration in from one-fourth to one-half the time. This finding was disproven by Boyes and Bunnell (17) in 1939 and in 1936 and 1940 by Bentley and Hill (10) all of whom found that there was no advantage in using predegenerated grafts. The latter transmitted axons just the same as, but no faster than, fresh grafts. Bentley and Hill found in addition that many axons did not travel down through old tubules but traveled anywhere within the nerve sheath Barnes and Baensch (18) in 1945 sutured the lower end of a useless ulnar nerve to its proximal nerve This was a predegenerated graft of 5½ months. It behaved microscopically just as would the lower segment of a nerve after direct suture and conducted axons well, even 113 days after the injury Sanders and Young (9) testing out predegenerated grafts on cats, found that the rate

to note in this connection that the only human nerve homografts to show any convincing evidence of recovery were three of Duel's 1033 six cases of facial nerve homografting. In all of these the dosage of tissue was low, the nerve transplanted was of small diameter and the gap to be bridged was short.

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of recovery was just the same as with fresh grafts. The graft itself seemed to be a little firmer.

## AUTOGENOUS NERVE GRAFTS

### History

Duroux (10) compiled a chronological list of those early investigators who reported the use of nerve grafts regardless of kind although mostly heterografts and homografts were used. Albert of Wein in 1876 and 1881 was the first to report and his was a homograft. Then followed Landerer in 1886, Mayo Robson in 1888 and 1890, Atkinson and Ward in 1888. In the next decade reports appeared from Mayll, Gerourg and Harrison in 1892, from Moultin in 1893, from Bradley and Dean in 1896 and the earliest autograft was described by Ferguson and Lange in 1899. The new century was opened by a report from Mitchell in 1902. He was followed by

TABLE 1

*Results of autografts in man in which the state of recovery is variously reported*

| Author and Date | Total Number of Cases | Number Improved | Number Un-improved |
|---|---|---|---|
| Cohen (1917) | 1 | 1 | — |
| Eden (1917) | 2 | — | 2 |
| Dean (1906) | 1 | 1 | — |
| Schmidt (191 ) | 3 | 3 | — |
| Charrier (1918) | 2 | — | 2 |
| Gosset, Thomas and Levy Valensi (1919) | 2 | 2 | — |
| Soutter and Twining (1918) | 3 | 2 | 1 |
| Auvray (1919) | 1 | 1 | — |
| Copland (1920) | 3 | 3 | — |
| Swan (1919) | 2 | 2 | — |
| Joyce (1919–1920) | 7 | 5 | 2 |
| Gosset and Charrier (1922) | 20 | 16 | 4 |
| Stookey (1922) | 1 | 1 | — |
| Frazier (1920) | 7 | 2 | 5 |
| Delageniere and Thuel (1924) | 3 | 3 | — |
| Bunnell (1927) | 4 | 4 | — |
| Tinker and Tinker (1937) | 3 | 3 | — |
| Foerster (1931) | 19 | 17 | 2 |
| Bunnell and Boyes (1939) | 17 | 1 | — |
| Total | 101 | | |

TABLE 2

*Results of autografts in man in which the state of recovery is more carefully reported*

| Author and Date | Total Number of Cases | Number Markedly Improved | Number Slightly Improved | Number Unimproved |
|---|---|---|---|---|
| Bunnell and Boyes (1939) | 17 | 13 | 4 | — |
| Foerster (1935) | 19 | 5 | 12 | 2 |
| Swan (1919) | 2 | 2 | — | — |
| Joyce (1919–1920) | 7 | — | 5 | 2 |
| Bunnell (1927) | 1 | 1 | — | — |
| Stookey (1922) | 4 | 4 | — | — |
| *Total* | 50 | 25 | 21 | 4 |

Heath in 1903, Peterson and Powers in 1904, Durantl, Rowley and Bacher in 1905, and by Kennedy, Ballance and Mayll. Duroux concluded that good results were obtained.

Sanders (8) has made an exhaustive study of the history of autografts and has tabulated the data presented (table 1). His figures showed 82.2 per cent of patients improved but being dissatisfied with the documentation, Sanders tabulated data on 50 more carefully described cases. Of these 46 showed recovery and 25, marked recovery. All of them involved thin grafts which were nourished through and through.

An article by Platt and Bristow (20) published in England in 1924 was far reaching in discouraging the use of nerve autografts. The authors presented here the results of grafts done in World War I; all cases resulted in failure. As these grafts (in 18 cases) were tubularized with fascia and the tube was injected with olive oil or petrolatum, they cannot be considered as autografts at all. But nerve grafts were then in bad repute until 1932.

Then from 1932 to 1936 came the elaborate articles by Ballance and Duel with many photographs of faces in symmetry in repose and actively moving. No detailed reports were available. The cases of decompression, nerve suture and nerve grafts were so mixed up that one was left with the impression that some of the nerve grafts were successful. Tickle carried on their work.

Stimulated by this work many surgeons have joined the facial nerve by autograft — notably Cawthorne in 1937 in 3 patients, Kisch during the same year in 2 patients who improved. Mc

Arthur in 1938 in one patient who improved and Collier who in 1940 had 15 patients 9 of whom improved. All of them, including Ballance and Duel, used predegenerated grafts, but these have proved to be unnecessary

In 1936 Duel stated that of 87 facial nerves operated upon he had done 77 and Tickle, 10 These included decompressions. A collection of 17 postoperative photographs was shown. Of these, 12 were of nerve grafts, 10 showed facial action, and 2 symmetry

Others who have grafted the slender facial nerve successfully are Martin, 1931 Bunnell 1930, (in 4 cases, 3 with good results and 1 lost) Hamben Thomas, 1934 Sullivan (21) 1934 one case McArthur (22) one case Foster (23) 1935 two cases Cardwell, 1938 Seely 1947 Horgan (24) 1940 one case resulting in active motion not complete Maxwell (25) 1951 4 cases of extratemporal graft to the facial nerve one resulting in good action, one in symmetry and 2 in slight motion

Bunnell and Boyes in 1939 published a series of 32 autogenous nerve grafts with very good results. These also were of slender nerves, as they were of the hand, and in the forearm there were cable grafts.

Under the impetus of success with slender nerve grafts, a great deal of research was done during and after World War II Under the leadership of Seddon of the Oxford Center and his colleagues, who early made a detailed tabulation of results, there was an enthusiastic revival of interest in nerve grafts. A firm foundation was laid by close microscopic study laboratory experiments on animals and by very careful follow-up on the clinical cases. Hetero- and homografts were discarded for man and autografts were recognized as a proven success. Outstanding workers in this field are Seddon Sanders Barnes, Gutmann Holmes, Bentley and Hill, Young Medawar and Strange.

## Contributions to Nerve Grafting

### *Contributions by Ballance and by Duel*

The work of Ballance (26–29) and Duel (30–38) on the facial nerve was an early stimulus for the use of autografts. Facial nerves were operated on in 09 baboons, and in 40 nerve grafts were placed between the divided ends of the facial nerve. Activation of the faces was reported On May 18 1931 the first nerve graft of these authors was done in a human patient, a 27 mm. graft from Bell's nerve in a baby From that time up to 1936 both Ballance and Duel published many articles. Duel reported 50 cases of facial paralysis including 8 grafts in 1934 and 77 cases of facial paralysis in general in 1936 In March of the latter year he reported a hundred cases of facial paralysis in which he himself operated upon 77 patients and Tickle on the remainder Among these were 20 cases of grafts done in suppurating wounds. As a presentation of results from nerve grafts 10 patients were pictured showing the face in action. Several more showed symmetry in repose. A face showing action is a definite indication of a successful nerve graft. Since then Tickle has increased his list of facial nerve operations to 300 (39) but the results of the grafts are not given.

Sanders (8) stated that 'Ballance and Duel's series concerned small grafts but detailed statistics were not available. Decompression and graft cases were all mixed up The above is as near a tabulation of their results from nerve grafts as could be made from their articles.

The operation method used by Ballance and Duel was done in the presence of suppuration or granulation tissue The graft was merely laid between the two severed nerve ends the normal blood acting as the glue No attempt was made to suture A gold leaf was then laid on for several weeks and wet dressings were applied. They were changed daily or more often It must be said though that the best union cannot be obtained in the presence of infection and many of these cases could not be cleaned up in a reasonable time Conditions now however have changed with the use of antibiotics. Quite a few of the photographs of Ballance and Duel's patients showed voluntary activity thus proving the value of nerve grafts

### *Autografts by Karsten Kettel of Denmark*

Kettel (40 41) has carried out grafts in 69 cases, mostly of nerve grafts and a few of nerve sutures of the facial nerve mostly in the Fallopian canal, and has given a follow-up on all but two He used the ilioinguinal nerve for a graft as it is easy to locate and is of the right caliber He sutured only when it was feasible.

There was insufficient innervation in four patients one of whom had postoperative roentgen ray treatment, and in one there was no return innervation. In some grafting operations on the face Kettel could not find the distal branches Of

TABLE 3

*Results of nerve grafting by Karsten Kettel*

| | No Regeneration | Clinically Unsatisfactory Regeneration | Smile | Smile with Closed Eye | Smile, close Eye and Wrinkle Forehead | In all |
|---|---|---|---|---|---|---|
| Nerve grafting | | | | | | |
| 1 Intratemporal | 1 | 3 | 4 | 18 | 5 | 31 |
| 2 Intra and extratemporal | 0 | 1 | 2 | 10 | 1 | 14 |
| 3 Extratemporal | 0 | 0 | 0 | 0 | 0 | 0 |
| Nerve suturing | | | | | | |
| 1 Intratemporal | 0 | 1 | 7 | 0 | 0 | 8 |
| 2 Extratemporal | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 *In all* | 1 | 7 | 35 | 6 | 6 | 53 |

53 cases, 45 of nerve graft and 8 of nerve suture whenever the muscles were not too degenerated and the operation was well done, there was a good result clinically that is, in 48 of the cases or 90 per cent the result was good (table 3)

The grafts gave even better results than the sutures The grafts varied from 5 to 50 mm. in length and the time since the injury from one week to a year It took from 4 to 21 months for signs of return innervation to appear the average being about 10 months Kettel's article is well substantiated by 36 postoperative photographs of patients with active movement of the face in 5 of which the forehead wrinkles

In America valuable contributions have been made by Davis Lyons, Woodhall Spurling Baensch and Tarlov

*Autografting by Bunnell*

*It has been shown that the early successes of* autografts were with slender nerves The author commenced repairing such nerves within the hand in 1918, doing several successful nerve grafts 2 of which were 6 inches long (42, 43) In 1927 repair of a series of a hundred of these tiny nerves was reported (44) including 6 cases of autograft and several instances of suturing the tiny motor branches of the median and ulnar nerves

Prior to 1925 there was nothing in the literature about suturing nerves below the wrist It was an easy step from this to suture another *tiny* nerve *the facial* within the temple bone (45) This happened to be the first time it was done and subsequently the author sutured the facial nerve in the temporal bone three times (46) rerouting it to overcome gaps These junctures were made in clean fields and carefully sutured by means of special curved needles only 3 mm. long, made by Shrimpten and Fletcher of England. *Emotional expression returned to the face in two of these patients the third was* a failure because of infection. The regeneration *of the facial nerve and nerves in the hand is* usually rapid and extensive probably because these nerves are near the periphery in the nervous system and because they are pure nerves either motor or sensory

On December 22, 1930 the first nerve graft was applied to the facial nerve a three-branched graft *2½ inches in length, from the sural nerve* from within the temporal bone out in the cheek. This graft resulted in symmetry and considerable active motion. In 21 months malignancy recurred, *and the whole block of tissue when examined* microscopically showed axons growing through it. A year later Ballance and Duel did their first *graft of the facial nerve*

In a paper on nerve grafts by Bunnell and *Boyes in 1929 (17), the results of experimental* grafting by Boyes of the sciatic nerve in cats were given. Boyes showed that slender grafts survive in the surrounding lymph, while large grafts show some necrosis in the center and good axon growth along the periphery The axons grew almost as rapidly as after the nerve suture There was no difference in the rate of growth of predegenerated grafts and undegenerated *grafts.*

Thirty-two autografts in hands in 21 patients of Bunnell over a period of 15 years were reported, *the areas of anesthesia and their recession* according to postoperative time being charted for each graft The results were uniformly good and convincing and compared well with the results after nerve suture Accurate sheath suture was done with the finest silk and the degree of regeneration was *in direct proportion* to the accuracy of the juncture Many illogical methods of joining nerves were condemned. The sural nerve *found in the center of the calf* was usually used for the graft but occasionally one from the stump of an amputated finger or the palm when the finger had been amputated was

utilized. No patient complained of the minimal anesthesia resulting.

In three instances, 3- and 4-strand cable grafts were used. A 3-inch 4-ply graft in the radial nerve above the elbow gave good extension of the thumb and fingers but left out the extensors of the wrist. Two six-inch 3-ply cable grafts in the median nerve and a simultaneous graft in the radial nerve in one patient and the ulna in the other resulted in return of sensation to the entire area of anesthesia, which was large because two nerves were severed. In one patient a 1¼ inch graft from an amputated finger was used to bridge the motor branch of the ulnar nerve in the palm. In 20 months good active motion had returned to the adductor brevis of the thumb and the first interosseous muscle. There were three nerve grafts in the foot and a 2½ inch three-branched sural graft in the facial nerve.

All of the 32 nerve grafts were followed through to successful regeneration, and two additional cases which could not be followed were not included in the report. It was found that the degree of regeneration was in direct proportion to the accuracy of the juncture and that results are better from grafts to good tissue than in cicatrix.

Since these cases were reported 76 more autografts have been made in 37 patients, making a total of 108 autografts in 60 patients. For the most part no extensive follow ups were made on these cases, as confidence in the effectiveness of autografts in hands had already been established. Also some of the patients were Army and Navy personnel who were not available later. Among the cases however 20 autografts in 15 patients were followed. There were 4 cases of multiple grafts for the facial nerve extratemporally. All of these were of multiple strands of sural nerve on the side of the face. One Navy patient was lost and another with a tumor of the parotid gland, died from a cerebral complication. Two other patients gained good control of the facial muscles.

One patient, as the result of an automobile accident four months prior to surgery had total right facial paralysis. The side of the face had been lifted from the bones upward as a flap. On September 7 1950 the main facial nerve and all its peripheral branches were dissected out from the dense scar tissue. There was a defect of 1½ inches in length. This was filled by five nerve grafts from the great auricular nerve the nerve being split. The patient first noticed motion about the mouth and chin in April. In May eight months postoperatively, the face was symmetrical and the patient could whistle—something which he could not do before. The upper lip twitched, the right angle of the mouth could be moved ¾ of an inch and four wrinkles appeared when the patient retracted the angle of his mouth. He was not available for further follow-up.

Another patient, in whom the ear was torn off and the side of the face avulsed, showed total facial palsy. Two months after his accident a 2-ply 3-inch graft from the sural nerve was sutured to the trunk of the facial nerve at the stylomastoid foramen and to the five peripheral branches in the face, which were joined in two groups. Voluntary motion started in eight months and was quite good in 1½ years. All parts of the face from the lower eyelid to the platysma muscle were functioning well. The activation of the forehead did not return so a fascial sling was used to keep the eyebrow elevated. The patient had good emotional expression.

In these cases where the tiny peripheral ends of the facial nerve were dissected out and finally joined to the graft by sutures and in several cases where the motor nerves within the hand were sutured it was found that there is no nerve so small that its function will not return if it can be joined by suture. In about 15 cases in which the whole parotid gland was excised, every tiny branch of the facial nerve was preserved. This made a fine lace-work of nerve function across the face. In every case there was facial paralysis for three months, then function gradually returned throughout. These tiny nerves were much like nerve grafts as their blood supply across the face was temporarily destroyed and had to be replaced before function returned.

In one case the median nerve at the elbow was severed in two places 2½ inches apart. The severed ends were sutured. In a year sensation returned throughout but not opposition of the thumb. In another patient the median nerve was sutured and a 2 inch gap in the sensory radial nerve was filled by a sural graft. In a year sensation to pin and light touch was present throughout the hand even though two adjoining nerves had been severed. In a case of severe electric burn of each hand in one arm a 7½-inch graft

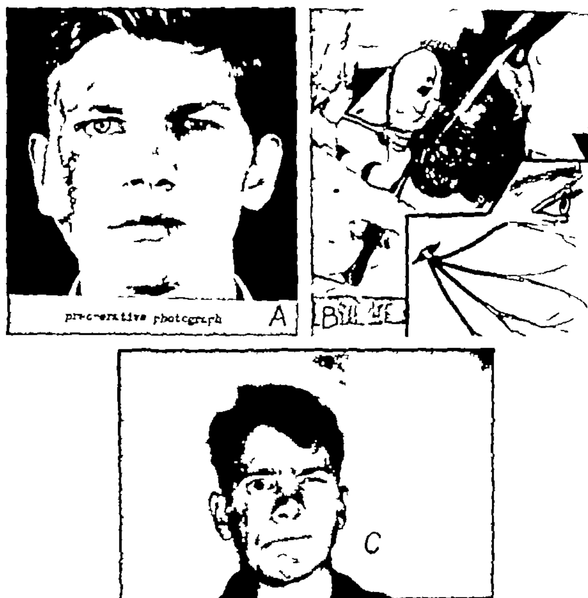



FIG 117 Case J R. C A From an automobile accident June 23 1950 a flap of face including the facial nerve was avulsed and the zygoma maxilla and mandible were fractured Complete facial palsy resulted.

B On September 7 1950 the facial nerve at the foramen spinosum was joined to the distal branches in the parotid gland by four 1½ inch strands of free grafts from the sural nerve The branch above the lower eyelid had been totally destroyed.

C Fourteen months later symmetry and definite motion of the face was restored He whistled the *upper lip twitched and the angle of the mouth moved* ¾ inches making four wrinkles in the face

of the ulnar nerve split and opened like a ribbon joined the median nerve to its three branches in the hand. In the other hand a 6-inch graft of sural nerve joined the ulnar nerve to its two branches and a similar one joined the median nerve to its four branches In two years sensation returned throughout

In a case of anesthetic long, ring and little fingers three 1 inch grafts of sural nerve were used to join the ulnar nerve to the three digital nerves in the palm. *In seven months sensation* returned throughout including the isolated area of the little finger *A cotton gin was the* cause of evulsion of the digital nerves from the palm. The gaps were filled *by four sural nerve* graft ranging from 1½ to 3 inches long In two years *sensation had returned to all area* except one side of a distal segment in each of two fingers *In the remaining cases that were followed* through there were various length of graft



FIG 118 A.

FIG 118 A. Case L R. *Left* On April 21 1953 a tractor rolled over the patient tearing off the ear and avulsing the left side of the face forwards destroying the branches of the facial nerve and fracturing the mandible Preoperative appearance

*Center* On June 17 1953 the jaw was pinned and by two 3-inch strands of sural nerve the stub of the facial nerve was joined to its six distal branches. An artificial ear was furnished. In 8 months there was activation about the chin In 10 months there was activation in the left cheek In 15 months there was symmetry and all the face moved except the eyebrow and forehead In 20 months there was emotional expression in the whole face except above the palpebral fissure A fascial sling was placed to hold up the forehead and the lids were sutured together in the outer angle *Right* Appearance at 20 months

B Distribution of facial nerve



FIG. 118 B.

from the sural nerve used to fill gaps in the different nerves of the hand, and sensation returned at the usual rate and to the same degree as described above

*Autografts by Loyal Davis*

Testing the theory that the connective tissue scar formed at the line of suture between the distal end of a nerve transplant and the end of the distal segment of the peripheral nerve may constitute a formidable barrier to the downgrowth of axons, Davis (47) experimented on four groups of dogs. He resected the suture line scar and resutured this juncture of nerve ends at 45 60 70 and 80 days. He believed his experiments show that the scar may be a barrier through which the nerve fibers cannot pass and that resection and resuture of the juncture will permit continued downgrowth of the axons into the distal segment of the nerve On the other hand cicatrix in the distal juncture has been denied by Sanders and Young as the only collagen producers are the cells of Schwann and the juncture occurs before the axons arrive

*Autografts by Sanders and Young*

Two-centimeter autografts of posterior tibial nerve into peroneal nerves of rabbits were made by Sanders and Young (9). The junctures were made by plasma. In 15 to 25 days the graft was exposed and it was determined how far the axons had grown by pinching the nerve from the distal end upward until pain was felt. The graft was then examined microscopically.

A total of 63 grafts was tested. Reversed polarity made no difference. The graft united and continued to live. In 15 days fibers were present in the graft and in 25 days they had passed the distal juncture and had grown 41.7 mm. from the upper juncture, that is 21.7 mm. into the peripheral stump. New fibers grew through the graft only slightly more slowly than through a normal peripheral stump (2 mm. a day compared to 3.9 mm. and with a latent period of 9.2 days to cross the distal juncture compared with 8 days; later this latent period was found to be practically nil—1.7 days). Wallerian degeneration and regeneration were the same as in the normal peripheral stumps. Some microphages remove the myelin and axons. Unlike the situation with homografts, lymphocytic infiltration was usually absent.

Both junctures were quickly made by Schwann cells coming from both the graft and the host. There was no connective tissue and the axons when they eventually arrived quickly crossed the distal juncture, thus negating the claim of *Davis and Cleveland that the distal juncture* should be resutured. In 25 days fibers were becoming medulated. There was good blood supply down the nerve itself and no central necrosis in the small nerves. Repeated experiments showed that predegenerated grafts had no advantage over fresh grafts except that they were slightly firmer.

Extensive investigation was also made of homografts and heterografts; the findings have been described under previous headings.

Klar (48) in 1943 reported 21 autografts. Of these five showed movement of every muscle, five showed action of at least one muscle, and four cases were too recent for evaluation of result. The diameters of the grafts were less than those of the nerves.

Many others have obtained good result from autograft. Christensen (49) in 1951 bridged a gap in the radial nerve above the elbow by two strands of sural nerve and obtained extension of the wrist, fingers and thumb. In 1918 Björkesten (6), after using 37 heterografts with negative result, made 25 autografts, 6 nerve flaps, 3 delayed flaps, 4 cable grafts and 13 straight autografts. The flap grafts were of course unsuccessful. Björkesten reported considerable improvement in half of the patients and some improvement in 21.

Marble (50) in 1953 reported 5 cases of sural autografts in the hand with good result. Titrud bridged a 4.5-cm. gap of radial nerve with two strands of sensory nerve. In 4½ years the wrist extended to almost normal, the fingers would straighten, and there was slight extension of the thumb. Graham reported in *History of World War II* that he had made 24 autografts with good functional results, especially when the surrounding tissues were not too cicatricial.

Frackleton reported also in *History of World War II* that he had made 7 autografts in the hand with the return of sensation in 5 of them. He used two or three strands of sural nerve in 5 patients for gaps of 5 and 6 inches in median and ulnar nerves. Patients were followed for only 7, 6 and 5 months. Return of function *had not started by then. At 7 months a second* suture was done at the distal end. Microscopically nerve fibers were found traversing the juncture. Littler in the same volume reported two autografts in the ulnar nerve. Return of sensation to the terminal segment in 5 and 7 months was reported. A touch at the tip of the finger was referred to the base.

In 1945 Stoneman (unpublished) at Letterman Army Hospital grafted an ulnar nerve with its blood supply into the median nerve. First the lower ends of the ulnar and median nerves were sutured together and the ulnar nerve was cut off high. When Tinel's sign had climbed up the ulnar nerve, this nerve, now having blood supply from the stump of the median nerve, was swung down to be sutured to the peripheral stump of the median nerve. Sensation returned to the area supplied by the median nerve. Wrong pointing was present and the anesthetic area was obtained by blocking the median nerve with novacaine.

In 1947 Strange published a similar case and by 1950 gave the results in the high standard of details set by Seddon. The result was excellent even to a radial block which did not affect the

area of sensory return. Strange called this a pedicled nerve graft. The present author recently operated to restore opposition to the thumb and flexion to the proximal finger joints on a patient (at Letterman Army Hospital) who 1½ years ago had had a pedicled nerve graft of the ulnar nerve at the elbow placed into the median nerve. There was good return of sensation and some flexion of the muscles in the forearm. On blocking the radial nerve with novocaine, sensation left the back of the thumb cleft but did not leave the area supplied by the median nerve until that nerve was also blocked.

*Autografts by Seddon*

Seddon has led the way in improving follow-up standards of nerve repair (16, 51–55) and in changing the attitude toward nerve grafts from pessimism to optimism. In 1950 he reported on 58 autografts and showed that in over half the regeneration was as good as by direct suture, the rate of recovery of a sutured nerve being at first 3 mm. a day and then slowing to 1 mm. and 1.5 mm.

A nerve of the size of the popliteal nerve was found to be too large for a graft because of central necrosis. Having placed three strands of an internal cutaneous nerve as a cable graft, 7.5 cm. long, into the median nerve, Seddon removed it (51) 7 months later and did a direct suture. He found the condition excellent histologically. The Schwann cells were proliferating and the myelin sheaths were commencing to form. The Schwann cells proliferated from each of the four stumps, joining the nerve graft and constituting the bands of Bungner down through which the axons grow. The microscopic appearance of the graft was exactly like that of a normal peripheral stump which had been sutured.

In a very comprehensive report (52) in 1947 results of the work by Seddon and his colleagues at the Oxford Center in 1081 cases of nerve injury are given. Of these, 41.5 per cent required surgery and 8.6 per cent or 59 cases required nerve grafts. The results were presented in a carefully prepared and complete table in which the varying factors governing a given result can easily be seen.

"In twenty, 38.5 per cent, recovery was as good as that seen after the most satisfactory end-to-end suture of the same nerve at the same level." In 7 more cases recovery is progressing toward the same degree. Thus good results were obtained in 51.9 per cent of the cases. In 8 cases the recovery was partial but valuable. In 67.3 per cent the graft was worthwhile. Failures occurred in 17 cases or 32 per cent.

Since most of the patients had severe injuries the results must be considered good. Detracting factors were the long time lapse between injury and grafting, separation of the graft due to tension or infection, the inadequacy of resecting the peripheral stump, and poor nutrition and excessive cicatrix of the part. Stress is placed on the exactness of the juncture. Excellent technique must be credited to the operators in this series—Seddon, Zachary, and Ruth Bowden.

*Autografts by Holmes*

A nerve autograft, as stated by Holmes (56) is the same as a peripheral nerve stump except that it must regain a blood supply. This comes through each end and the intermediate tissues. If the latter is cicatricial, the graft suffers accordingly. The graft, therefore, should be placed in a good vascular bed of tissue. A segment of nerve grafted free in the tissue showed poor Wallerian degeneration, evidently because it was not nourished from each end. Both stumps should be good in order to nourish the graft. Schwann tubes are 15 $\mu$ in diameter and in 6 weeks 2 $\mu$, that is, they show degeneration of the endoneurium. This is largely dependent on vascularization. In cases of ischemic contraction, therefore, vascularization is poor and also in digits where blood vessels have been destroyed. It is a local process. A liberal resection of the distal nerve stump provides better vascularity.

*Autografts by Robert Martin*

In 1931 Martin (57) reported a direct suture within the temporal bone done in 1929 which gave good activation of the face. On August 3, 1939, (58) he grafted two strands of sural nerve, 1.5 cm. long, into a facial defect in the Fallopian canal with fairly good result. In 1933 (57) and 1936 Martin grafted part of the saphenous nerve into the facial nerve and obtained a good result in the lower two-thirds of the face. As indicated by news of work soon to be published, Martin has to date made 20 autografts of the facial nerve. Only one was a failure, which was due to the presence of infection. His other autografts were not carried out in the presence of infection,

and all patients recovered good motion except in a case involving the frontalis nerve. One case traced for 20 years showed, as did the others continuous improvement especially in facial expression.

### *Autografts by Conley*

Seven times on removal of a part or all of the parotid gland grafts from the opposite great auricular nerve were used to fill the gaps of the facial nerve in the face (59). In three patients in whom the grafts were from 50 to 90 cm long, from 65 to 90 per cent of function was obtained in from 8 to 12 months. In one case the result was poor and two others showed failure. Illustrations of excellent motion in one patient are shown even though plastic cylinders were placed around the distal junctures.

### *Autografts by Peer*

In 1931 Peer and Walker (60) and in 1955 Peer (61) reported the experimental findings in three human nerve grafts transplanted in contact with nerve and four such grafts transplanted in abdominal fat. The posterior cutaneous nerve was used in all of the patients.

When nerve grafts were transplanted in contact with nerve the axons and myelin sheaths degenerated soon after transplantation. The Schwann cells appeared viable up to about four days and then could not be identified among the large numbers of proliferating fibroblasts from the endoneurium. One cannot state that the Schwann cells died since dead or degenerating Schwann cells were not observed in any sections. The axons from the proximal host nerve attempted to grow down through unobstructed channels in the graft and these are associated with Schwann cells. Possibly some surviving Schwann cells in the graft participate in the process of repair.

Sections of nerve grafts buried in abdominal fat demonstrated that the fibroblasts in the supporting structure of the graft survive and undergo early proliferation (in three days). These fibroblasts however retained their characteristic arrangement, so that endoneurium could be differentiated from the epineurium and perineurium on microscopic examination seven months after transplantation. The axons and myelin showed progressive degeneration and eventually disappeared. The Schwann cells however appeared to survive the initial shock of transplantation and appeared viable up to three and four days. In later sections the Schwann cells could not be identified in the multitude of proliferating fibroblasts from the endoneurium. There is of course no axon replacement when nerve grafts are transplanted in abdominal fat.

## SUMMARY

*Heterografts* are valueless and merely irritating foreign bodies.

Homografts, though effective in rabbit and cats when short, soon undergo necrosis and fibrosis with blocking of the axons. They are worthless in human beings until some way is found to prevent the acquired immunity that destroys them.

Autografts are successful although the supply is very limited.

### History

Nerve grafting was first done with a homograft in 1870 by Albert of Wein, who was followed by others who used impractical methods. Between 1917 and 1939 one hundred autografts were reported but only fifty were sufficiently documented. Of these 92 per cent showed some recovery.

From 1927 to 1939 many cases of grafts applied to facial and hand nerves were reported, thus reviving optimism about the use of autograft. Hand nerves and facial nerves regenerate very well as they are peripheral in the nervous system and are no longer mixed nerves. From 1940 to the present much careful investigation has been done in England under the leadership of Seddon so that now the employment of autografts is on a firm basis.

### Success of Autografts

Success in autografting nerves is attested to by statistics—those concerning the facial nerve for example. Kettel had only five failures. Martin only one in 20 cases. In practically all of his 58 cases which were followed Bunnell had recovery in the small nerves of the hand. Seddon obtained as good results with nerve grafts as with the best nerve sutures in 38.5 per cent of 50 cases. Of his results 31.0 per cent were good and 67.3 per cent were worthwhile.

Statistics however do not tell the whole story as many grafts are made under unfavorable

circumstances. It may be said that if the interval between injury and repair is not too long if the tissues are vascular free from cicatrix and the limb has good nourishment, if repair by grafting has been technically correct, if the graft is not too thick and no undue traction has been applied, a good regeneration will always take place.

### Criteria for Evaluation of Results

Careful documentation is essential to clarify whether improvement after nerve grafting is the result of regeneration or overlap and special tests are also required. When a nerve is severed, an area of anesthesia may be produced As a natural process it recedes somewhat from overlap of adjoining nerves that take over the function and as this happens there is some paresthesia. This type of recovery has its limits, however which become apparent when a large series of nerves severed and repaired by suture or graft are observed.

If after grafting, the area of anesthesia recedes at the same rate and to the same degree as it would after nerve suture, one has strong evidence that the graft is successful If then either the nerve of the graft or an adjoining nerve that could give overlap is blocked by novocaine, the degree of recovery from anesthesia due to the graft can be ascertained After a nerve is sutured or repaired by graft, there is considerable cross-crossing of the fibers that leads to wrong pointing If with closed eyes the patient errs in pointing to the spot of a pin-prick regeneration is indicated, but if he points correctly it is probably due to overlap In the case of a facial nerve graft, if symmetry and active motion return to the face the success can, of course, be ascribed to the graft.

Accurate records should be kept, both preoperatively and at intervals postoperatively As recommended by the Medical Research Council (1942) muscle action may well be recorded on a numerical scale as follows 0 no action 1 flicker 2, movement but not against gravity 3 movement against gravity 4 movement against gravity and some resistance 5 normal strength of movement. For sensory records light touch heavy touch, and pin prick should be drawn out accurately Data on two-point discrimination, Tinel s sign, and the area of paresthesia are useful. A further refinement is delineation of the area of sweating by the galvanometer or Quinizarin dye test, but these are time consuming procedures without sufficient reward.

Since even the best nerve suture does not yield perfect results, limitations must be expected of grafts. The following pertinent comment is quoted from Kettel Judge results not by how much the function falls short of 100 per cent but by what has been achieved compared to the condition that would have been present without surgical intervention.'

### Microscopic Aspects

When a nerve is severed the axons of the upper stump split into many The nerve end becomes capped over by fibrous tissue and as axons in their attempt to force through become entangled, a swollen neuroma forms. From those axons that escape through into the tissue Tinel s sign or tingling on tapping results.

The lower or peripheral stump for the first month swells from Wallerian degeneration due to proliferation of the cells of Schwann. Myelin and axons fragment and are taken up by macrophages. The cells of Schwann form into the bands of Bungner but retain their lumina for reception of axons The lumina gradually diminish in caliber from 15 to 2 $\mu$ in six weeks (Holmes)

When a peripheral nerve stump or a nerve graft is joined to the proximal stump the axons grow down through the tubules of the bands of Bungner at the rate of 2 mm a day in grafts and 3 5 mm. a day in peripheral stumps. Some axons also travel between these bands and within the nerve sheath. Motor and proprioceptive axons are relatively large sensory axons are smaller However all are able to insinuate themselves down through the Schwann cells even if their canals have become small.

When a nerve graft is placed each end heals rapidly by Schwann cells from both sides of the juncture. Therefore cicatrix is not present, and when the axons reach the distal suture line they pass through readily with hardly a pause. Consequently secondary suturing of the distal juncture is unnecessary It has been shown that there is no chemotactic action to lead the axons down the nerves

A nerve graft becomes vascularized through its two ends and rapidly forms a longitudinal blood supply It also receives blood vessels along its length. A graft laid in the tissues however, without its ends joined does not do well as the

intermediate vascularization is not sufficient. Similarly a graft with the ends joined but laid through scar tissue does not receive in its intermediate portion sufficient nourishment.

A dead or heterogenous nerve graft does not undergo Wallerian degeneration, which is an active process, and a homograft undergoes such degeneration until the active acquired immunity stops the process. As axons grow down they are at first non-myelinated, but as the cells of Schwann multiply these cells and the axons produce myelin. Non-myelinated fibers give Tinel's sign. An autograft acquires exactly the same appearance microscopically as a normally regenerating peripheral stump.

## Overcoming Gaps

Effort to approximate nerve ends should be made before resorting to nerve grafts. This consists of first freeing the nerve to beyond the joint below and the joint above and flexing these joints. To gain more slack the freeing can be further and more joints can be flexed. Next comes the transposition. The ulnar nerve may be freed well up the arm and down the forearm, stripping up and preserving its branches. It is then brought forward in the cleavage plane between the deep and condylar muscles. Similarly the median nerve is brought forward from the pronator teres and made to span the flexed elbow, and the radial nerve is similarly made to span the elbow. The facial nerve can be rerouted in the hypotenuse of the triangle. To repair a nerve in the arm or leg, the elbow or knee must be flexed. Nerves are so important that it is occasionally necessary to bend the limb at a fracture site.

Flexion must be maintained for four weeks, not three, as the juncture may part. Gradual extension twice a week should then be done but not by wedged casts or turn buckles since these sooner or later put the nerve under tension, rendering it ischemic. Extension should be done by the snub method. Metal splints are used that check or snub extension only, leaving the joints free to flex whenever an ache is felt in the limb. Thus it is possible to overcome gaps even up to 5 inches (14 cm.) long in each of the three nerves of the arm. In these extension should be made over a period of at least two months. Other methods are by bulb suture or shortening of bones. Nerves do not stretch, they grow longer.

The limits of gaps that can be overcome as set by other authors are: Naffziger, 2 to 3 cm.; Stiles and Forrester Brown, 1 to 2.5 cm.; Babcock, 3 to 7 cm.; Swan, 3 cm.; Bristow, $1\frac{1}{2}$ inches median and 6 inches ulnar; and Seddon, 10 cm.

## Sources of Autografts

It is difficult to find sufficient material for a gap in a large nerve without sacrificing some important function. Sometimes a nerve may be obtained from an amputation stump or if there are two nerves, the one of lesser importance may be taken. Usually for a nerve of the size of the median one, it is necessary to use a cable graft of three to four ply from some sensory nerve. The accumulated diameter should at least equal that of the host nerve and the length should be ample since grafts shrink a little.

Relative merits of available sensory nerves, which are all 2 to 3 mm. in diameter, the radial and sural nerves being the thickest, are shown as follows:

*Sural*: 25 to 40 cm. long, not much branching, negligible anesthesia resulting; found in mid calf beneath sural vein beneath deep fascia in upper half but superficial to it in the lower half. Graft of choice.

*Radial sensory branch*: 15 to 25 cm. long, not much branching, tendency to give tender neuroma; anesthesia greatly increased if branch is taken for the median nerve.

*Internal cutaneous*: 20 to 27 cm. long, branched.

*Long saphenous*: 25 to 40 cm. long, gives some anesthesia.

*Anterior crural*: 8 cm. long, too branched.

*Small sciatic*: 1 to 7 cm. long, too branched.

For the fingers a graft from a palm with a finger stump or from an amputated finger may be available, and for the facial nerve, the sural or great auricular nerve may be used.

A graft may be used from either a sensory or motor nerve and placed in either direction. A large nerve graft like the popliteal nerve becomes necrotic in the center from lack of nourishment, the axons being seen in the periphery. A small graft becomes nourished through and through. Thus a cable graft of several small strands is also thoroughly nourished. A piece of ulnar nerve, if slit and opened out like a ribbon, becomes nourished. Both ulnar and median nerves have succeeded as graft, but the median nerve is a little thicker than is desirable.

### Technique

The degree of regeneration is in proportion to the accuracy of the juncture. The approximation in the author's opinion, is more accurate and better able to stand tension if done with 7-0 silk on a Weldon corneal eye needle than with plasma glue. The former are used as interrupted sheath sutures placed with exquisite care. A hand-sewn juncture insures against inversion of part of the sheath and against emergence of axons into the tissues, which would result in Tinel's sign and detract from the regeneration. Often individual bundles can be recognized and approximated accurately by the first silk suture in the sheath and as each part of the whole circumference is sutured separately the accuracy can be exact. Plasma glue is apt to melt if placed in puddled serum, hematoma or infection.

Infection disposes to inaccurate nerve junctures and interferes due to the production of scar tissue. Although in the early facial nerve grafts reported there was definite regeneration, the results would probably have been better if infection had not been present. Martin did his grafting in clean areas and Kettel under antibiotics, both with good results.

It is not necessary to resuture the lower juncture of a nerve graft for the reason given above (microscopic aspects) No nerve is too small to regenerate if it can be sutured even if but one suture is used. This applies to the peripheral branches in the face and the two motor branches within the hand.

To suture a cable graft, first one stitch is taken in the sheath in each of the several strands. As this is tied it groups the strands together Then the sheath sutures are placed all around, just as in ordinary nerve suture.

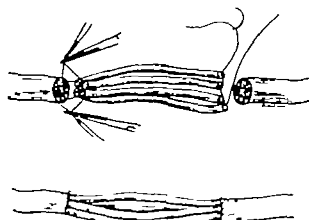


Fig 110 Technique of suturing a cable graft First one sheath suture of 7-0 silk groups the strands together Then an ordinary sheath suture is done

Before a nerve is lifted from its bed, its exact rotation should be marked with a silk suture on the uppermost surface of each end. The nerve graft should not be twisted if so motor fibers will grow down sensory pathways and *vice versa* and so be lost.

Nerve ends should be cut back liberally until good nerve bundles are present without scar tissue between them. Such an area is usually at the neck of the neuroma, but cutting back should be done more amply in the peripheral stump If nerves are cut off with a knife and frayed by tremor of the operator they will so lose their consistency as to make suturing difficult and inaccurate. A clean cut with sharp scissors separates the fibers the least.

It is exceedingly important to provide a bed of good vascular tissue for a nerve graft. If a graft is placed in cicatrix, scar grows into the graft. The nerve should be detoured around the cicatrix. A little extra length of the graft does no harm A cicatrix may be replaced by a pedicled skin graft. Tubulization to keep out scar should never be done because it interferes with the healing and keeps out blood supply None but exact end-to-end junctures should be made. Predegenerated grafts have no advantage over fresh ones.

A *pedicled nerve graft as described above* insures a good result, since good vascularity is maintained.

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Infection disposes to inaccurate nerve junctures and interferes due to the production of scar tissue. Although in the early facial nerve grafts reported there was definite regeneration, the results would probably have been better if infection had not been present. Martin did his grafting in clean areas and Kettel, under antibiotics both with good results.

It is not necessary to resuture the lower juncture of a nerve graft for the reason given above (microscopic aspects) No nerve is too small to regenerate if it can be sutured even if but one suture is used. This applies to the peripheral branches in the face and the two motor branches within the hand

To suture a cable graft, first one stitch is taken in the sheath in each of the several strands. As this is tied it groups the strands together Then the sheath sutures are placed all around just as in ordinary nerve suture.

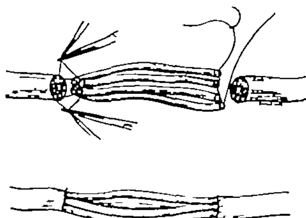


Fig 110 Technique of suturing a cable graft First one sheath suture of 7-0 silk groups the strands together Then an ordinary sheath suture is done

Before a nerve is lifted from its bed, its exact rotation should be marked with a silk suture on the uppermost surface of each end. The nerve graft should not be twisted if so motor fibers will grow down sensory pathways and *vice versa* and so be lost.

Nerve ends should be cut back liberally until good nerve bundles are present without scar tissue between them. Such an area is usually at the neck of the neuroma, but cutting back should be done more amply in the peripheral stump If nerves are cut off with a knife and frayed by tremor of the operator they will so lose their consistency as to make suturing difficult and inaccurate. A clean cut with sharp scissors separates the fibers the least.

It is exceedingly important to provide a bed of good vascular tissue for a nerve graft. If a graft is placed in cicatrix, scar grows into the graft. The nerve should be detoured around the cicatrix. A little extra length of the graft does no harm. A cicatrix may be replaced by a pedicled skin graft. Tubulization to keep out scar should never be done because it interferes with the healing and keeps out blood supply None but exact end-to-end junctures should be made. Predegenerated grafts have no advantage over fresh ones.

A pedicled nerve graft as described above insures a good result, since good vascularity is maintained.

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intermediate vascularization is not sufficient. Similarly a graft with the ends joined but laid through scar tissue does not receive in its intermediate portion sufficient nourishment

A dead or heterogenous nerve graft does not undergo Wallerian degeneration which is an active process and a homograft undergoes such degeneration until the active acquired immunity stops the process. As axons grow down they are at first non myelinated but as the cells of Schwann multiply these cells and the axons produce myelin. Non-myelinated fibers give Tinel's sign. An autograft acquires exactly the same appearance microscopically as a normally regenerating peripheral stump

### Overcoming Gaps

Effort to approximate nerve ends should be made before resorting to nerve grafts. This consists of first freeing the nerve to beyond the joint below and the joint above and flexing these joints. To gain more slack the freeing can be further and more joints can be flexed. Next comes the transposition. The ulnar nerve may be freed well up the arm and down the forearm, stripping up and preserving its branches. It is then brought forward in the cleavage plane between the deep and condylar muscles. Similarly the median nerve is brought forward from the pronator teres and made to span the flexed elbow and the radial nerve is similarly made to span the elbow The facial nerve can be rerouted in the hypotenuse of the triangle To repair a nerve in the arm or leg, the elbow or knee must be flexed Nerves are so important that it is occasionally necessary to bend the limb at a fracture site.

Flexion must be maintained for four weeks not three as the juncture may part. Gradual extension twice a week should then be done but not by wedged casts or turn buckles since these sooner or later put the nerve under tension rendering it ischemic Extension should be done by the snub method. Metal splints are used that check or snub extension only leaving the joints free to flex whenever an ache is felt in the limb Thus it is possible to overcome gaps even up to 5 inches (14 cm.) long in each of the three nerves of the arm In these extension should be made over a period of at least two month Other methods are by bulb suture or shortening of bones Nerves do not stretch they grow longer

The limits of gaps that can be overcome as set by other authors are Naffziger 2 to 3 cm. Stiles and Forrester Brown, 1 to 2.5 cm. Babcock, 3 to 7 cm. Swan, 3 cm. Bristow $1\frac{1}{2}$ inches median and 6 inches ulnar and Seddon, 10 cm

### Sources of Autografts

It is difficult to find sufficient material for a gap in a large nerve without sacrificing some important function. Sometimes a nerve may be obtained from an amputation stump or if there are two nerves, the one of lower importance may be taken. Usually for a nerve of the size of the median one it is necessary to use a cable graft of three to four ply from some sensory nerve. The accumulated diameter should at least equal that of the host nerve, and the length should be ample since grafts shrink a little.

Relative merits of available sensory nerves which are all 2 to 3 mm in diameter the radial and sural nerves being the thickest are shown as follows

*Sural* 25 to 40 cm long not much branching negligible anesthesia resulting found in mid calf beneath sural vein beneath deep fascia in upper half but superficial to it in the lower half Graft of choice

*Radial sensory branch* 15 to 25 cm. long not much branching tendency to give tender neuromas anesthesia greatly increased if branch is taken for the median nerve

*Internal cutaneous* 20 to 27 cm long branched

*Long saphenous* 25 to 40 cm long gives some anesthesia

*Anterior crural* 8 cm long too branched

*Small sciatic* 1 to 7 cm long too branched

For the fingers a graft from a palm with a finger stump or from an amputated finger may be available, and for the facial nerve the sural or great auricular nerve may be used

A graft may be used from either a sensory or motor nerve and placed in either direction A large nerve graft like the popliteal nerve becomes necrotic in the center from lack of nourishment, the axons being seen in the periphery A small graft becomes nourished through and through. Thus a cable graft of several small strands is also thoroughly nourished. A piece of ulnar nerve if slit and opened out like a ribbon, becomes nourished. Both ulnar and median nerves have succeeded as graft but the median nerve is a little thicker than is desirable

### Technique

The degree of regeneration is in proportion to the accuracy of the juncture. The approximation in the author's opinion, is more accurate and better able to stand tension if done with 7-0 silk on a Weldon corneal eye needle than with plasma glue. The former are used as interrupted sheath sutures placed with exquisite care. A hand-sewn juncture insures against inversion of part of the sheath and against emergence of axons into the tissues, which would result in Tinel's sign and detract from the regeneration. Often individual bundles can be recognized and approximated accurately by the first silk suture in the sheath and as each part of the whole circumference is sutured separately the accuracy can be exact. Plasma glue is apt to melt if placed in puddled serum, hematoma, or infection

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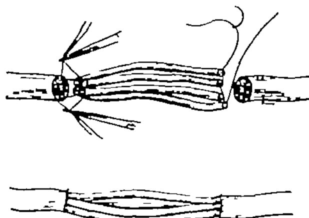


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PART VI

# *Teeth*

CHAPTER 9*

# *Transplantation of Teeth*

HAROLD S FLEMING

## Introduction

The aim of this chapter is to present and review work done in the field of the transplantation of teeth. This work can be considered under two major aspects, the *clinical* and the *experimental*. Reports of clinical experiences greatly outnumber those in the field of experimental research. Transplantation of teeth has been carried on for many centuries while records of investigative or experimental research date back only 200 years. In fact, some initial surgical practices had their origin in or evolved from the transplantation of teeth. In addition dental prosthesis began in association with these procedures. From transplanting and replanting of teeth early techniques were developed which led to the original ideas or concepts of tissue transplantation.

## DEFINITION OF TERMS

The numerous references to the clinical aspects of transplanting teeth fall into three categories—*replantation* or *reimplantation* *implantation*, and *transplantation*. Replantation and reimplantation usually denote reinsertion of teeth into their alveolar sockets following either intentional or traumatic removal or displacement. Implantation generally indicates the insertion of teeth into sockets especially prepared to receive natural or artificial replacements Transplantation is sometimes used in a less specific manner but usually denotes transferring teeth from one location to another in the oral cavity of the same individual or from one person to another The word explantation is commonly used in connection with *in vitro* studies. Frequently these terms are used interchangeably transplantation being the word most commonly employed.

Experimental transplantation has been employed to study the growth and development of teeth in most instances the tooth germs or developing teeth of laboratory animals have been used. There are two main divisions of experimental work, studies *in vivo* and those *in vitro* † *In vivo* deals with transplantation to living animals while *in vitro* explantation indicates cultivation in media away from the body Since the middle of the eighteenth century transplantation of teeth has been carried out experimentally in animals. Tissue culture techniques were not used until recently and recorded experiences with culturing teeth or tooth germs in nutritive media outside the body do not date back more than 30 years.

*In vivo* experimental transplantations of teeth or tooth germs have generally been made to locations outside the oral cavity and are grouped under the three categories *autogenous* *homologous* and *heterologous* A major part of this experimental work has been with autogenous transfers, consisting of transplanting teeth or tooth germs to new locations in the same animal. Homologous transfers denote transplanting between animals of the same strain or species heterologous transfers indicate transplantation between different strains or species. Considerably more work has been done with autogenous and homologous

* Preparation of this chapter was aided by a grant from the Department of Health Education and Welfare National Institutes of Health Bethesda Maryland

† Two reports of *in ovo* cultivation of tooth germs are included under the *in vitro* heading (1 2)

transfers than with heterologous ones. Some investigators use the terms heterologous and homologous in a broader sense than others. Various experimental animals including cats, dogs, rats, mice, sheep, goats, chickens, guinea pigs, rabbits, hamsters and salamanders have been used. The following sites have been utilized for transplantation of teeth or tooth germs: muscle, spleen, marrow cavities of long bones, prepared cavities in bones (including the jaws), ovaries, subcutaneous tissue, kidneys, alveolar sockets, peritoneal cavities, brains, eyes and cocks' combs.

A small number of investigators have worked with *in vitro* explants of tooth germs. In this aspect of experimental work, tooth tissues have been cultivated in hanging drop slides, watch glasses, roller flasks and Carrel flasks. Plasma, serum, and several types of synthetic fluids have been used as cultivating media. Thus far, *in vitro* studies have been concerned with survival, differentiation and organization of tooth germs and the relationship of epithelial tissues to mesenchymal tissues.

The chorio-allantoic membranes in hens' eggs have been used for *in ovo* cultivation. The discussion of this work is included in the section on *in vitro* studies.

## CLINICAL REVIEW

A number of references in medical literature cite case reports or clinical procedures concerning transplantation, implantation and replantation of teeth. Early medical literature indicates that these procedures were common. There is evidence that transplantation was practiced in ancient Egypt and later by the Etruscans, Greeks and Romans. For many centuries the Arabs practiced transplantation, and several historians state that the Arabs obtained this knowledge from the ancient Greeks and early Chinese. However, written accounts of such procedures from these early times are rare.

Indian skulls of pre-Columbian North and South America reveal instances of tooth transplantation and replacement of teeth by stones. Occasionally, reimplanted teeth filled with gold discs, jade, etc., were found. A root tip, partially amputated and presumably trimmed before transplantation, was discovered in the mandible of one of these old skulls.

Albulcasis de Condue (930–1013) (3) an surgeon, is the first individual to be credited with teaching that missing or lost teeth might be replaced. Little can be found in the literature describing the transplantation of teeth until the 16th century. However, in Europe and England references are made to barber-surgeons and surgeons who, in addition to their other duties, functioned as dentists, primarily extracting and implanting teeth. It was during the latter part of the reign of Henry VIII (1509–1547) that a royal decree prohibited barber-surgeons from performing any surgical operations other than extracting and implanting of teeth; henceforth in England, blood letting, etc., could be performed only by surgeons. At this time such a restriction was not placed on barber-surgeons on the continent of Europe.

From the sixteenth to the twentieth century transplantation of teeth had successive periods of popularity and neglect. Operators who reported their experiences during these periods were never in agreement on the procedures.

In 1786 Pierre Fauchard (4) reviewed the reports of Ambroise Paré, a Parisian barber (1510–1590), and others who had stated that "transported" teeth remained firm and solid, and Fauchard agreed that they served all the functions of the original teeth. He held that incisors, canines and pre-molars could be successfully transferred from one person to another if the tooth were immediately placed in the new socket before the blood had coagulated. He also recommended that persons providing the teeth be young and healthy. When human teeth were not obtainable, he suggested using the teeth of animals (trimmed to fit) and of beef cattle in particular. The use of animal teeth still had its advocates as late as 1898.

There were, of course, those who did not agree that transplanted teeth would remain firm in the alveolar process. These men often supported their opinion by citing unsuccessful instances of transplanting teeth.*

John Hunter (1728–1793) (5) popularized the replanting and transplanting of teeth during the middle and latter part of the eighteenth century. Although Hunter lived 200 years later, there was a great similarity between his teachings and

* David (6) mentions the following who believed that this operation was impossible and caused necrosis, fistulas and inflammation: Lartal [illegible], Bx (1725), Beedmoore [illegible], Courtin [illegible], Laforgue [illegible], Serrel [illegible], Carrel and Oudet [illegible].

those of Paré Hunter advocated the transplantation of teeth from one person to another, stating, "Success of this operation is founded on the disposition of all living substances to unite when brought in contact with one another, although they are of different structure and even though the circulation is carried on in one of them. Thus he recognized the importance of physiologic function in tooth transplantations. The procedures that he favored varied according to the success or failure of his most recent transplants. Sometimes he advocated boiling an extracted tooth before replacing it and tying it in place with silk or seaweed. He gave the term scion tooth to the tooth to be transplanted and suggested that all dentists have a stock of dead teeth on hand.

In addition to his clinical work, Hunter did experimental transplantation of teeth which will be considered later James Palmer (7) took exception to Hunter's opinions and disputed the importance of the relationship of vascularity to a transplanted tooth. Also in a critical review of Hunter's work C Vasey (8) stated that success of transplantation depended upon the presence of cementum and of a partially developed root. He felt that it was impossible therefore to transplant a fully developed tooth successfully

Dentistry in colonial America consisted, to a large extent, of extracting and replanting teeth which at that time was a highly remunerative occupation. Harvard Dental School and a dental school in Philadelphia were founded by men who were distinguished for their transplantation operations. The well known Josiah Flagg (1764-1816) of Worcester Massachusetts, was one of several highly regarded men taught by the itinerant French dentist, LeMaire. Gardette, (9) a contemporary transplanter of teeth and a critic of LeMaire wrote None of the teeth transplanted by Mr LeMaire in Philadelphia remained firm two years and in two or three cases that I have seen of teeth transplanted by other dentists, they did not remain firm one year He continued My opinion, therefore is that teeth cannot be transplanted from one mouth into another so as to answer the intended effect that is that the transplanted tooth will not become as firm and as useful as the one it has replaced "

Transplantations or *reimplantations* were usually performed when a tooth was decayed, painful knocked out or extracted by mistake There were also some practitioners who treated diseases of the gums by removing teeth and later reimplanting them. It was customary then as it is now, to treat teeth before replacing them. Frequently months elapsed before treatment was completed and the teeth were replaced. This treatment consisted of trimming away carious portions of the tooth and filling the root canal with gold, lead, or other materials It was advocated that teeth be soaked in special solutions, such as perchlorate of soda or bichloride of mercury before reinsertion. Often teeth were heated or boiled to kill the 'worms' before replanting. Another practice to prevent infections was the thorough drying of the extracted tooth for several months before replacement. Sometimes a wire was passed through or around the tooth and attached to adjacent teeth for retention.

Following reimplantation of removed teeth or transplantation of new teeth there followed an expected 'sickness" which lasted from four to six weeks. Considerable pain usually persisted until a well established fistula drained purulent material from the root apex area. Several treatises have been written on the care of patients during this critical period, since the transplant was often lost and sometimes the patient was also Reports of success varied greatly some operators claiming 100 per cent success while others indicated they had only partial success. Reimplantations that lasted for at least a year were considered successful. At times some lasted for as long as three or four years and infrequently for as long as five years. The transplantation of teeth from one individual to another began to lose favor again in this country as well as in Europe towards the end of the eighteenth centruy when it was found that communicable diseases such as syphilis and tuberculosis were sometimes transmitted in this manner

During the latter part of the nineteenth century clinical reports on transplantation of teeth became more numerous The majority of these reports considered such operations satisfactory * A few were against these procedures

* Theophilus David (1877) (11) wrote in *Etude Sur La Greffe Dentaire* on survival times for these transplants and of claims of certain operators Fauchard (4) who claimed 1 year Joux Mitscherlich (10A) Dopp, Magitot (12) Hunter (5) who claimed 4 years Bourdet who claimed 6 years and Pfaff and Taft who noted survival times of 10 to 16 years

and wrote accordingly others attempted to distinguish between favorable and unfavorable circumstances under which transplantation of teeth might be carried out Opinions differed concerning the importance of the periodontal membrane. Some maintained its preservation was essential for successful transplants William Younger (1869) (10) of San Francisco described an operative procedure that was for a time, universally accepted.* He was interested in reparative processes following transplantation and proposed that success or failure depended on regeneration of the periodontal membrane.† This approach is strikingly similar to the opinions of Vasey who lived about 70 years earlier

Younger's ideas were later expanded by Wilkinson (13) who was interested in absorption of grafts and who found that where the periodontal membrane remained intact, there was a more rapid union between the transplants and the hosts. Wilkinson's findings were also consistent with those of Vasey (8) It was Fletcher (14) who in 1891 advanced the idea that for complete success the periodontium must be intact, and the transplant must be made before the pericementum dies However there were men who proposed the antithesis of this Fletcher stated that one operator (unidentified) advocated stripping the periosteum from the tooth before transplantation. About 1878 Colemen of Guy's Hospital, London, was interested in spreading the practice of transplanting teeth which he claimed had fallen into disrepute. At this time there was disagreement and confusion concerning the conditions under which the transplantation of teeth should be attempted.

From the time of Fletcher and Younger (10) to the present the majority of reports on the clinical reimplantation of teeth have been confined to single case histories In some instances there have been more than two or three cases reported by a single practitioner but very few representative series have ever been presented. One exception was Bugnot (1880) (15) who reported on 20 cases Bugnot gave this subject considerable thought he advocated that better success might be obtained by transplanting embryonic teeth and hoped that this would some day be done between individuals of different species

Ottolengui (1880) (16) wrote on the origin of the transplantation operation and cited Hunter's use of the cock's comb. It has been noted that the cock's comb as a result of Hunter's work, was considered an ideal place to keep teeth prior to retransplantation since at this site the fibers of the periodontium could be kept viable He pointed out the environmental differences between this site and the oral cavity This work is of interest because it presents several drawings of the operative procedures as well as the instruments used. From this time to the present, despite a few variations in technical procedures, the cases reported on reimplanted teeth afford little more than routine information.

Hipple (1890) (17) who favored implantation of teeth summed up opinions of his era and suggested conditions under which implantation of teeth could be carried out successfully these ideas however were not new

It should be emphasized that despite improvements in instruments and the advantages of x ray studies as well as improved histologic method little has been basically changed in techniques from the times of Hunter Fauchard Paré and David to the present (1958) In addition there has been no increase in the duration of these reimplantation ‡ Naturally there has been, however a better understanding of the biologic and pathologic events following reimplantation.

## Recent Literature on Transplantation of Teeth

Kromer (1948) (18) pointed out that originally replantation was carried out on a purely empirical

* This operation consisted of making an artificial socket in the bone A linear incision was made through the gum and soft tissues on the labial and lingual sides the periosteum was dissected away with a sharp chisel—care being taken to retain the tissue in place so that a flap could be made to hold the tooth A socket was made with a set of trephines and burrs and the bone was reamed out to the dimensions of the root The pulp of the tooth was then drawn through the apex the canal filled with gutta percha and the apex sealed with gold Before insertion the tooth was heated for 15 to 20 minutes at 108 to 110 F in 1 1,000 bichloride of mercury

† This opinion is held by many today

‡ Perint (19) stated in 1949 From 158 cases of reimplantation results remained satisfactory in 61.2 per cent of the cases after one year 52 7 per cent after 2 years 41 1 per cent after 3 years 37 3 per cent after 4 years 35 4 per cent after 5 years 31 0 per cent after 6 years 19 0 per cent after 7 years 8 1 per cent after 8 years 4 4 per cent after 9 years 2 5 per cent after 10 years

basis and not until the recent clarification of the histologic processes involved was this procedure placed on a solid scientific basis. The value or importance of preserving the periodontal membrane is still debated. As Kromer wrote, some believe that re-attachment of the tooth in the socket is due to scar contraction. Others have shown the existence of an organic connection, and a few have said that the periodontal membrane goes into regressive metamorphosis. Kromer however, like most clinicians, believes that special care should be given the periodontal membrane. He recommends the use of antibiotics and handling of the tooth so that the fibers cannot dry out nor receive any chemical or physical injury. An auxiliary device for holding the tooth to be reimplanted and also keeping it moist was designed by Kromer.

It has been demonstrated that denuding a small part of the root surface of the periodontium results in a metaplasia of the cementum following reimplantation. As a result dentin of the tooth root may eventually be replaced by either connective tissue or osteoid tissue or both and the crown, which is all that finally remains, ultimately falls off.

Operators have no greater success today than they did formerly with the duration of transplants. The majority last for only two to four years; infrequently some remain in place for longer periods. A more conservative outlook was recently expressed by Lovel and Hopper (1954) (20) who stated that the duration of success may range from several months to several years. Although success may be limited, there are certain advantages to the removal and reimplantation of teeth. The infected alveolar area can be more adequately treated, and in addition root canal treatment and apiectomy can be carried out more efficiently than when the tooth is *in situ*.

The transplantation of unerupted teeth or of tooth germs to new locations within the mouths of patients has recently received considerable publicity. This procedure is now an important part of oral surgery, for it is often considered feasible to autotransplant teeth or their anlagen to areas in patients' mouths where teeth are missing or have been lost. Results of these procedures are far more gratifying than simple reimplantation. Apfel (1953) (21) recommends that this procedure should be performed with non-functional teeth having incompletely formed roots. He considers that young persons in their teens are best suited for such operations. This particular technique calls for deep imbedding within the bone, so that the transplanted teeth may erupt normally and come into occlusion through their own direction. When tooth germs or buds are used, they are carefully removed in their follicles from their crypts and retransplanted to new locations. In most instances transplants so handled have vital pulpal areas and erupt normally with an intact periodontal attachment to the alveolar bone.




FIG. 120. Avulsed and reimplanted teeth. *1* and *2*. Photograph and x-ray taken in 1945 five years following reimplantation of upper left central incisor. Root had been filled and tooth reimplanted within a few hours after being avulsed. X-ray shows resorption of root. In 1950 the tooth exfoliated. *1'* and *2'*. Photograph and x-ray taken in 1932 of upper left incisor which was avulsed and root fractured. Root was not treated. *3'* and *4'*. Photograph and x-ray of same tooth in 1936. In 1955 tooth was still healthy and firm. (Courtesy of Dr. B. G. Anderson, Dept. of Surgery, School of Medicine, Yale University.)

Experimental tran plantation in lower animals has shown repeatedly that those tooth germs which grow and develop best have demon trable nerve fibers and patent blood vessel within the pulpal areas In addition when pulpal areas remain intact the morphology of the transplant is retained. These points are cited since in the first stages of tooth development, blood vessels and nerves do not have as important a relation ship as they do following eruption and matur ation.

Myers (1954) (22) suggests that more experi mental work is necessary before it can be determined under what conditions teeth can be succes fully transplanted. He estimates that in the not too distant future tooth banks will be utilized this may be possible as soon as storage method are worked out. Myers ideas differ little



FIG 121 Clinical tooth transplant *1* Peri apical involvement of a devitalized mandibular left first molar in an 18 year old girl Al o shown (in part) i an impacted third molar on the ame side Third molar in position of the first molar after the latter wa extracted and a bed had been prepared for the transplant The transplant was fixed in place with tainless steel wire and wa placed approximately 0 5 mm out of occlu ion The procedure wa *carried out in October 19 1*

*(Contin d on F g 122 )*



FIG 122 Clinical tooth transplant *3* X ray of tooth in place In February 1955 Vitality wa tested with a Burton Vitalometer *4* Oral photograph taken February 1955 (Courtesy of Prof J R Hayward Oral Surgery Department School of Dentistry University of Michigan )

from those of Younger (1891) who suggested that viable teeth be kept on hand for tran planting

## GROWTH AND DEVELOPMENT OF TEETH *IN SITU*

For better comprehension of their biologic behavior following transplantation normal or expected growth and development of tooth germs should be reviewed briefly A comprehen ion of the processes involved may lead to explanation of the success or failure of tran plant of teeth or of tooth germs. Such comprehension i also fundamental to a knowledge of when the component cell of tooth germ may or may *not* exhibit normal behavior following transplantation.

Development of teeth starts with organization of proliferations of epithelial cell having a spe-

Current studies in the author laboratory are determining at what tage of development teeth can best be tran planted and under what condi tion they can be kept viable until used *Vascu larization i a major problem* Once thi can be solved tran plant can be routinely made

cific positional association with other collections of mesenchymal cells within the oral cavity. Odontogenesis of human teeth begins during the sixth week of embryonic life with the proliferation and invagination of the oral epithelium covering the maxilla and mandible just inside the lip furrow band of each jaw. Thus a solid strip of epithelial cells called the dentinal lamina is formed. Following their initial organization these specialized cells must rapidly pass through a number of concurrent, overlapping changes in order that normal mature tooth structures may be formed. For this future normal development of teeth, organization as well as proliferation of ameloblasts and odontoblasts must occur in a specific anatomic relationship to each other. Development of teeth depends on a regular sequence of these morphologic and histologic changes that take place or are induced. These progressive developmental events are appropriately described as the *bud*, *cap* and *bell* stages. In human beings symmetrical proliferations at ten points in each lamina give rise to the buds of the deciduous teeth. It is from a proliferation of the basal cells in these buds that development of the deciduous teeth and first permanent molars is induced. Later posterior extensions of the dental lamina give rise to the anlagen of the remaining permanent molars. By ten to twelve weeks anlagen of the remaining permanent teeth begin to form from lingual extensions of the buds of their deciduous predecessors. These specific collections of epithelial cells are called *enamel knots* and each forms an inner and outer layer from which *enamel organs* are formed. The behavior of these inner and outer epithelial layers is important for future normal development of the teeth. The outer layers attain a definite cellular arrangement and become known as the outer enamel epithelium. The inner layers form the inner enamel epithelium or ameloblasts. As these layers separate from each other an area forms between them that is composed of a loose stellate arrangement of cells. These areas are known as the enamel pulps which are large originally yet enlarge continuously until the crowns of the teeth begin developing and ultimately occupy these spaces. The cap and later the bell stage become evident as cells of the buds extend outward and downward respectively. As adjacent mesenchymal tissue becomes enclosed by proliferations of these epithelial cells, the dental papillae are formed. Cells of the inner enamel epithelium form basal columnar layers adjacent to cells of the mesenchyme. By differentiation these cells form ameloblasts, and opposite them a single layer of mesenchymal cells, which will soon form the odontoblasts now organize. As these ameloblasts become functional they elongate and become palisaded. Their nuclei become polar and mitotic division ceases. The dental papillae have now been enclosed by proliferations of the specialized epithelial cells and the morphology of the teeth now becomes evident. By this time the ameloblasts located at the cusp-tips will start production of the enamel matrix against the basement membrane, separating the enamel organs from the dental papillae. At this time the dentinal matrix begins to form opposite the young enamel. Extensions of enamel formation outline the entire crowns of the teeth. Now the dental papillae become organized into the dental pulps.

Relationships of these organizational and developmental changes to environmental conditions are important, for energy requirements of the teeth at certain times during development of the embryo and fetus are apt to assume precedence over the demands of all other organs. Thus, for future normal growth the teeth at specific times during development have higher energy requirements than other organs at that particular time. Developmental anomalies result from interference at such times. Once the initial activities of particular cells have utilized stored glycogen then availability and transport of energy producing substances are important. It has been observed that by increasing sucrose in the diets of rats the calcium and phosphorous content of incisor teeth is elevated. In fact, it has also been mentioned and emphasized by R. L. Hartles that these sucrose induced effects are confined to developing teeth and not to bone. This would further indicate the necessity for an abundant source of energy during enamel formation.

## *IN VITRO* STUDIES

Tissue culture will be covered in more detail in other sections but as an introduction to the cultivation of tooth germs in artificial media it is necessary to review briefly these transplantation methods. While the cultivation of tooth germs in artificial media dates back only to 1925 tissue culture was first demonstrated by Roux in 1885 (23). Later Harrison (1907) (21) established the

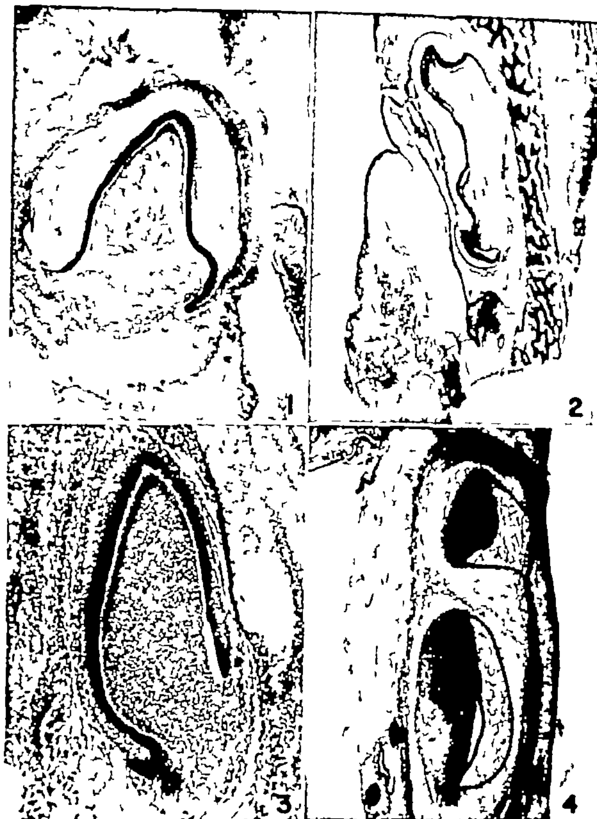



FIG. 123. Tooth germ controls. *1.* Lower deciduous canine tooth germ from 4 month human fetus ×32. *2.* Lower deciduous second molar tooth germ and anlage of 6 year molar from 1½ month human fetus ×3[illegible]. *3.* Lower incisor tooth germ from 25 day guinea pig embryo ×[illegible]. *4.* Lower molar tooth germ from 30 day cat embryo ×101. (*1* and *3* reprinted by permission of the editors of the Journal of Dental Research.)

fact that under a suitable environment living cells could be maintained and would function outside the living donor organism. Problems of asepsis and of the proper nutritive media have always been obstacles or problems in tissue culture. *In vivo* transplants are automatically protected by the natural defenses of the living animal against possible contamination. They likewise can receive adequate nourishment from their hosts. Tissues removed from these natural body defenses to tissue cultures are helpless against infection by bacteria. They must also be able to sustain themselves on or in the specially prepared media or tissue juices. In recent years antibiotics have been advantageously employed to maintain asepsis.

It was at first considered significant that groups of certain cells maintained themselves in *in vitro* cultivation. Later it was observed that cells so explanted actually underwent mitotic division, with the number of newly formed cells outnumbering those that degenerated. There were, of course, misconstrued ideas concerning what actually could be accomplished in tissue culture. The following statement was made concerning tissue culture of teeth in a widely read scientific journal 15 years ago. "If human tooth germ cells were available, from these microscopic specks entire human teeth could be synthesized in the test tube—the main factor being merely the appropriate nutrient mixture."

While many persons have cultivated tissues *in vitro*, there have been only about a dozen persons who have studied tooth germs or teeth in this manner. The work of these investigators is presented and reviewed chronologically.

Yumikura (1925) (25) wished to establish the relationship of odontoblasts to cementoblasts since cementum was often found in the dental pulp. This *in vitro* investigation was carried out 10 years prior to Glasstone's original work. Rabbits ranging in age from 2 to 5 months were used. For reasons of asepsis, teeth were removed laterally through the buccal or labial wall of the mandible and not directly into the oral cavity. Both the hard and soft parts of the teeth were explanted as well as scrapings of cells from the enamel organ, the pulpal area, and the periodontium. Tissues to be explanted were first washed in Ringer's solution and then transferred to culture flasks. The culture medium was made from auto- or homeoplastic blood plasma and Ringer's solution. In addition, fresh blood plasma from other species of experimental animals was used. Periods of cultivation ranged from 3 days to 3 weeks.

It is interesting to note the results obtained with scrapings of odontoblasts. When these explanted odontoblasts were cultivated alone, they did much better than when connective tissue accompanied them. The presence of connective tissue appeared to have a toxic effect on the odontoblasts. When cultivated alone, the odontoblasts lost their Tomes' fibers but retained their cellular and nuclear structure. Yumikura did not state whether the cell walls continued to adhere to each other as Pinkerton and Boyle (1935) (26) observed in their tissue culture explants. Endothelial cells from capillaries often became confused with odontoblasts, but these cells were always fewer in number and small. From this work Yumikura concluded that the most highly differentiated cells degenerated first. Thus the odontoblasts appeared to be the most sensitive, the ameloblasts next, and the cementoblasts the least sensitive. If odontoblasts are considered the most highly specialized cells of the tooth germ, this conclusion is correct. This ordering is debatable, however, as some consider ameloblasts to be more highly specialized. Mitosis and proliferation were evident in the non-specialized cells, and it is presumed that in this instance Yumikura was referring to cells of the stratum intermedium. Odontoblasts at the root tip were also specifically mentioned as being subject to mitosis and proliferation, and it is possible that these cells at this location were not highly specialized. Connective tissue was most proliferative when cultivated in this manner. Cells of the outer enamel epithelium formed nests of cells and epithelial pearls. In addition, these epithelial cells grew more to a greater degree independently of connective tissue than with it. This was probably due to connective tissue outgrowing and crowding the other tissue. Growth was most extensive on the peripheries of the explants.

In 1929 and 1930 Fell and Robison (27) first produced evidence that bone retained the capacity to differentiate *in vitro*. The difference between histo- and chemo-differentiation was initially proven by Fell in a study on the mandibular skeleton of the fowl in relation to phosphatase activity. As a result of this research Fell pointed out to Glasstone (28) the possibility of cultivating tooth germs *in vitro*. The watch glass technique was used by Fell and was originally utilized by

Glasstone The procedure consisted of placing the explant on a plasma clot in a small watch glass which in turn was placed in a small covered Petri dish on a mat of cotton wool moistened with physiologic saline to prevent evaporation. Glasstone painted the under side of the watch glass black to facilitate observation.

This work by Glasstone was extremely important because it demonstrated that certain embryonic cells have an inherent capacity for self differentiation. Thus a tooth germ when removed from the rest of the body had a retained capacity for producing components of a developing tooth in a normal histologic manner The organizing influence of one group of cells (ameloblasts) was also demonstrated Although Yumikura's report was much earlier Glasstone's report was the first on the actual cultivation of whole tooth germs by *in vitro* methods In this initial study 18- to 21-day-old molar tooth germs of rats were used.

Later Glasstone (29 30) used the cover glass and depression slide technique for another *in vitro* study and this time employed a different culture medium. The explants were transferred to fresh media every 72 hours instead of every 48 hours as with the previous study and much better results were obtained. The development of cusp formations in rat and rabbit molars was investigated in this latter work In still another study (31) by the cover glass technique Glasstone was able to demonstrate that halved molar tooth germs of rabbits retained their capacity for the same developmental pattern as whole tooth germs This capacity of rabbit molars persisted up to the twenty-second day of fetal life

Pinkerton and Boyle (26) in 1935 made a report on the *in vitro* cultivation of tissues They used tooth germs from newborn kittens and under aseptic conditions extirpated and explanted entire tooth germ which included enamel and dentin cusps as well as parts of the enamel organs and dental pulp They claimed that ectodermal and mesodermal tissues grew rapidly maintaining their histologic characteristics They also observed a metaplasia of cells of the enamel organ into a stratified squamous epithelium. This finding is strikingly similar to the earlier work of Yumikura and to the more recent studies of Szabo (32) It should be noted that these investigators also found after serial transplantation that cells from the odontoblastic layer retained the peculiar property of developing long cytoplasmic processes The latter observation was not confirmed by Szabo or Yumikura who observed a tendency for dedifferentiation of odontoblast in extirpated pulps. They did state however that no new enamel or dentin was formed.

Studitsky* (1930–1937) (2) used the chorio-allantoic membrane for the cultivation of pulps of tooth germs from 10-day-old dogs Explants were obtained from molars and premolars and were incubated for varying periods up to 10 days While the study was in a broad sense confirmatory of Glasstone's initial *in vitro* observations some ideas concerning the organizing influence of the epithelial cells of the tooth germ on the dental pulp and odontoblasts were advanced. This concept had been investigated *in vivo* by Huggins and his associates (33) and was later explored by Hahn (34) Studitsky suggested that non-specific epithelial cells might have an organizing effect on mesenchymal tissue and would later have a more precise effect on the pulp of the tooth germ. In support of these opinions previous studies on the development of bone by similar methods were cited. It should be remembered, however that the odontoblasts in the tooth germs that Studitsky used were at that stage of development where they had already been "labelled"

Nuckolls (1940) (35) cultivated unerupted molars of one-day-old rats *in vitro* and also transplanted these tooth germs to the brains of rats His observations are largely in agreement with those of other investigators for he reported that enamel epithelium has an organizing influence on the formation of dentin. Once it degenerates the morphology of the developing tooth is lost and dentin formation is irregular Nuckolls also spoke of the organization of a bony crypt by the tooth germ but it is not clear whether this occurred in the brain transplant or in *in vitro* cultivation. If the latter was the case this result is not in agreement with the findings of other investigators and may have resulted from the explanting of adjacent embryonic tissue with the tooth germ.

Losee (1943) (36) describes a method of growing rat tooth germs in a depression slide In the detailed presentation of this work the importance of obtaining aseptic material and maintaining th

* The work of Studitsky ( ) and the later work of Glasstone (1) are considered under this section although in a strict sense these reports should be entered under a separate heading i e The cultivation of tooth germs *in ovo*

asepsis is emphasized. First molar tooth germs from 20- to 21-day rat embryos were used. A cover glass technique similar to Glasstone's but with a deeper depression slide was used. This particular type of slide thus allowed the use of six drops of medium instead of two drops. The medium for each explant consisted of three drops of rabbit plasma and three drops of embryonic chick extract. Before replanting, which was done from 48 to 72 hours, tissues were washed with Tyrode's solution to remove degenerative products. Through these procedures a tooth germ in one instance, was kept 95 per cent viable for 78 days.*

Grobstein (1951) (37) cultivated embryonic shields isolated from 6- to 8-day mouse embryos. This was done in Carrel flasks for 4 to 5 days within plasma clots composed of chicken plasma chick embryo juice and Tyrode's horse serum. These explants were subsequently retransplanted to the anterior eye chambers of mice, and on one occasion a fully developed mouse incisor was observed in the recovered tissue.

From Lefkowitz and associates (1954) (38 38A, 39) come several recent reports on the cultivation of rat molar tooth germs *in vitro* These investigators have cultivated these explants "in the clot and are now making a comparison with results obtained on the clot. Carrel flasks were used and the culture medium was made from lyophilized chicken plasma combined with either rat or chicken embryo extract. It was postulated that viability of the cells was maintained at a better rate with rat embryo extract than with chicken embryo extract † Tooth germs were cultivated for varying periods up to 20 days. Apparently no attempts were made at replanting the explants, for in this study interest in survival and initial growth and development were foremost. As could be expected younger explants exhibited more growth than did older explants

The possibility in the future of obtaining growth stimulating substances by using heads of embryos for media was considered. It has been previously pointed out that the addition to culture media of traces of embryo extract from areas where the tissue was obtained in donor embryos increases the growth rate and probably the general activity of transplanted cells. It furthermore seems possible that such an extract may be more or less of a stimulant to growth of certain explants, depending upon the particular body part from which it is taken. Thus, use of the heads of embryos for tissue extracts may be a particularly effective stimulant in the culturing of tooth germs.

*In vitro* growth is a process that accompanies differentiation but is actually a separate mechanism. What may stimulate one type of tissue may not necessarily stimulate another As Lefkowitz and his colleagues pointed out the manifestation of growth is by cell division in the proliferative zone and this mitotic division of cells may be the only measure of *in vitro* growth for these explants. It is also true that the growth potential of tooth germs is inherent in certain fated cells within tooth germs. Thus, until suitable culture media are devised, no growth beyond a certain limited size may be obtained.

Recently Glasstone (1954) (1) studied the development of tooth germs on the chick chorio-allantoic membrane and made a comparison with her *in vitro* studies of mammalian tooth development. Under these conditions special reference was made to the formation of enamel and dentin. The tooth germs explanted were first and second molars of fetal rats, mice and golden hamsters. The tooth germs of rats and mice were taken from 18- to 21-day-old fetuses their stage of development was equivalent, according to the author to those obtained from hamster fetuses of 14 to 16 days The tooth germs were grafted to the chorio-allantoic membranes of hens eggs of 8 days incubation. Glasstone described in detail the cultivation of tooth germs in this manner and pointed out that tissues cannot be so cultivated for more than 11 days due to the birth of the chicks Development of enamel and dentin was evident but of greater significance was the demonstration of free red blood cells and blood vessels within the stellate reticulum of the enamel pulp ‡

* Much would have been added to this presentation had pictures and photomicrographs been included

† As did Loew Lefkowitz presented a detailed description of technical procedures which may be of value to others who are about to engage in the tissue culture of tooth germs Lefkowitz explained in detail how rat tooth germs can be obtained and how recovered explants should be oriented

‡ One of the advantages of *in ovo* cultivation is that the developing chick circulates blood within the transplant and this may account for the presence of blood vessels and red blood cells in the enamel pulp as observed by Glasstone

Amelogenesis begins with penetration of vessels

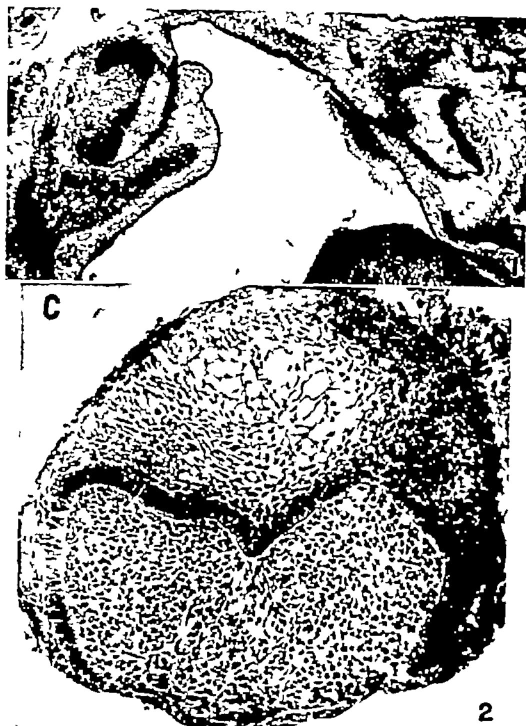



FIG. 121. *In vitro* cultivation. *1*, Molar tooth germs in 10 day-old rat *in situ*. *2*, Cultivated 10 day-old tooth germ dissected with follicular wall. Period of cultivation 8 days. Note advance in cusp outline, invagination of proliferating zones, fibrous invasion of central cell area, formation of predentin. *C*, Clot. *O*, Outgrowth. Section cut buccolingually. ×25. (Courtesy of Dr. William Lefkowitz. Reprinted by permission of the editors of the Journal of Dental Research.)

Stodtky, who much earlier and in a similar manner had explanted tooth germs from neonatal pups, did not allow them to develop on the chorio-allantoic membrane for more than 10 days. No actual comparison can be made of the results obtained by these two investigators, since they used tissues at dissimilar stages of development from different animals.

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through the enamel pulp to the stratum intermedium in the developing molar teeth of the albino rat.




Fig 125 Sections through hamster molars *1* Longitudinal section of the first and second molars at 15 days The cusps are present in the first molar but not in the second ×32

*2* Longitudinal section of a 15-day molar grafted for 10 days Notice continuous layers of dentin and enamel The latter is the thicker and appears darker ×58

*3* Longitudinal section through 15-day second molar grafted for a further 10 days Cusps odontoblasts and thin layers of dentin and enamel have formed Capillaries and erythrocytes can be seen in the enamel organ over the right cusp The host blood vessels are massed round the outside of the tooth germ ×66

*4* Section of a control molar tooth of a hamster one day after birth to show the early stages of invasion of the stellate reticulum by capillaries and erythrocytes The enamel is just beginning to form ×141

*5* Section of a 15-day second molar grafted for 10 days, to show the early invasion of the stellate reticulum by erythrocytes as in the previous figure There is a thin layer of darkly stained enamel ×141

*a* ameloblasts *b.v* blood vessels *c.a* chorio allantoic membrane *d* dentin *e* enamel *o.e.e* outer enamel epithelium *er* erythrocytes *o* odontoblasts *o.e* oral epithelium *p* pulp *s i* stratum intermedium *s r* stellate reticulum (Courtesy of Dr Shirley Glasstone Reprinted by permission of the editors of the Journal of Anatomy Cambridge England )

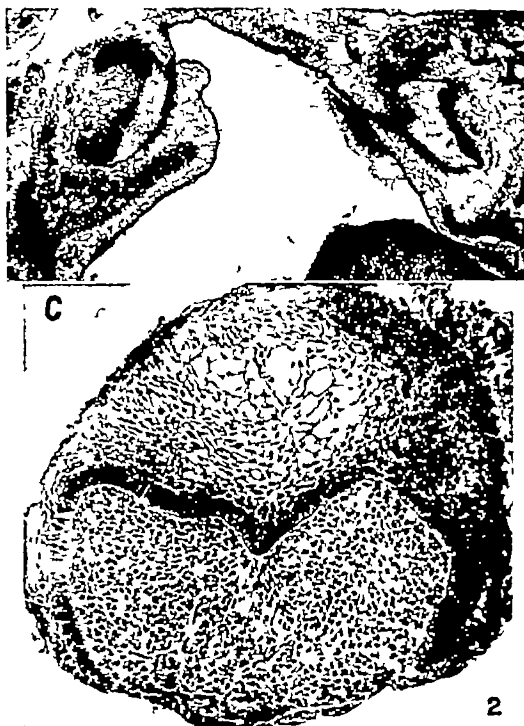



FIG 121 *In vitro* cultivation *1* Molar tooth germs in 10-day-old rat *in situ* *2* Cultivated 19 day-old tooth germ dissected with follicular wall Period of cultivation 8 days Note advance in cusp outline invagination of proliferating zones fibrous invasion of central cell area formation of predentin C Clot *O* Outgrowth Section cut buccolingually ×225 (Courtesy of Dr William Lefkowitz Reprinted by permission of the editors of the Journal of Dental Research )

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through the enamel pulp to the stratum intermedium in the developing molar teeth of the albino rat




FIG 125 Sections through hamster molars *1* Longitudinal section of the first and second molars at 15 days The cusps are present in the first molar but not in the second ×32

*2* Longitudinal section of a 15-day molar grafted for 10 days Notice continuous layers of dentin and enamel The latter is the thicker and appears darker ×58.

*3* Longitudinal section through 15-day second molar grafted for a further 10 days Cusps odontoblasts and thin layers of dentin and enamel have formed Capillaries and erythrocytes can be seen in the enamel organ over the right cusp The host blood vessels are massed round the outside of the tooth germ. ×58

*4* Section of a control molar tooth of a hamster one day after birth to show the early stages of invasion of the stellate reticulum by capillaries and erythrocytes The enamel is just beginning to form ×141

*5* Section of a 15-day second molar grafted for 10 days to show the early invasion of the stellate reticulum by erythrocytes as in the previous figure There is a thin layer of darkly stained enamel ×141

*a* ameloblasts *b.v* blood vessels *c.a* chorio-allantoic membrane *d* dentin *e* enamel *o.e.e* outer enamel epithelium *er* erythrocytes *o* odontoblasts *o.e* oral epithelium *p* pulp *s.i* stratum intermedium. *s.r* stellate reticulum (Courtesy of Dr Shirley Glasstone Reprinted by permission of the editors of the Journal of Anatomy Cambridge England )

Szabo's *in vitro* work (1954) (32) is of considerable interest for the study he made of outgrowths from the explants of teeth. He used the hanging drop method as well as the roller flask method of cultivation and also employed several kinds of media. It is interesting to note that streptomycin was used in the media for the elimination of bacterial contamination. Szabo explanted unerupted molars and erupted incisors of the lower jaws of white mice and was unable to culture enamel organs of the unerupted molars successfully. It was previously pointed out that the outgrowths from extirpated explanted pulps were fibroblastic. These cells became flattened and lost their shape although they did maintain a tendency to adhere to one another by their cellular walls. By this fact and also because they always grew out from the same part of the incisor Szabo identified these outgrowths as odontoblasts. The internal pulp changed in accordance with age, and collagenous fibers replaced the cellular elements.* Szabo raised the question of the capacity of tooth germ explants to form calcified tissues *in vitro*.† From his studies it is observed that there is a tendency towards unorganized growth with a dedifferentiation of tissues. While these adaptive changes of epithelial tissues and migrations of pulp tissues were observed for periods up to three weeks, it would be interesting to follow the behavior of such tissues in a larger number of *in vitro* transplantations over much longer periods.

## Summary

In a recapitulation of *in vitro* studies of tooth explants several important facts emerge. To date no investigator has been able to produce a medium that will supply the nutritive elements necessary for calcification of these explants. There is general agreement among investigators concerning the organizing influence of ameloblasts after differentiation on the subjacent mesenchymal tissue. The fate of ameloblasts was similar in most of the experiments in which they were studied. Opinions vary, however, concerning the ultimate end of the odontoblasts.

The tooth germs that were explanted ordinarily came from embryos just before birth and in some cases were obtained from newborn animals. Comparison of results is difficult, however, because experimental conditions varied. The work of Szabo is significant because it may suggest means whereby teeth may be kept viable for long periods before transplantation. Losee was able to keep a rat molar viable for 78 days, the longest period yet reported under tissue culture methods. Lefkowitz has been painstaking in his techniques and his technical contributions should be of assistance to the neophyte in the cultivation of tooth germs. The classic work of Glasstone is the major contribution to the actual cultivation of tooth germs in artificial media.

Many of these tissue culture experiments with tooth germs should be repeated under identical conditions for further confirmation of results. By means of tissue culture, teeth or tooth germs may be stored and kept viable for indefinite periods, and ultimately transplanted to human beings. If a medium which will supply all the necessary nutritive elements can be found, fully calcified mature teeth might be developed by tissue culture methods.

## *IN VIVO* STUDIES

*In vivo* transplantation of teeth dates back to the time of John Hunter (1728–1793) (5) and perhaps earlier. Hunter reported the transplantation of a young cock's spur to its comb, stating that this type of transplant was well known at that time and had been frequently carried out. He extended his investigations by making other types of successful autogenous transfers. As a result of his experiments he concluded that the cock's comb was a favorable site for transplantation and accordingly utilized it for human tooth transplants. In his own words, "I took a sound tooth from a person's head, made a pretty deep wound with a lancet into the thick part of a cock's comb and pressed the fang of the tooth into this wound and fastened it with threads passed through other parts of the comb." Several months later the cock was killed, the head severed and injected with a minute injection. He continued, "I slit the comb and tooth into halves in the long direction of the tooth. I found the

* This observation is consistent with findings in *in vivo* studies of the dental pulp in the author's laboratory. Szabo noted the invasion by connective tissue of the enamel organ when it had been damaged during explantation. This phenomenon was also observed with tooth germ transplants *in vivo* and as a result of this invasion the enamel organ broke down.

† This problem has similarly confronted all investigators of *in vitro* tooth cultivation.

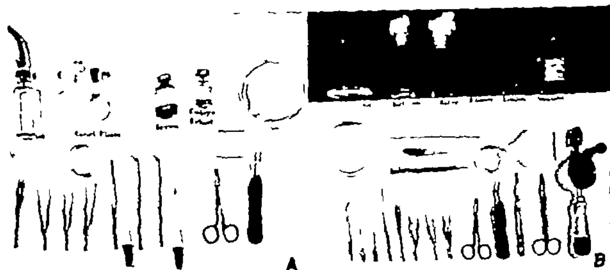


FIG 126 Instruments used for transplantation at the author's laboratory A *In vitro* equipment B *In vivo* equipment

vessels of the tooth well injected and also observed the external surface of the tooth with its socket Palmer (1835) (7) who reviewed Hunter's work, differed as to the importance and significance of blood vessels. While his explanations may not be clear Hunter stands out for his initial report on heterologous transfers of tooth.

About a century later (1860) Philipeaux (40) successfully transplanted a lower incisor of a newborn guinea pig to a cock's comb This transplant was allowed to remain in the comb for ten months during which time it grew from 8 to 13 mm. in length and also increased in diameter Philipeaux cited the experiments of Bert (41) Hunter (5) and Cooper, who likewise dealt with heterologous transplantations. Philipeaux's work while not original confirmed and extended the previous investigations of Hunter and Bert.

Mitscherlich (1863) (10A) performed experiments with a dog one year of age The second lower molar was extracted and reimplanted Six months later the animal was killed, the head cut off and the carotids injected. Examination of the tooth and comparison with the one opposite showed normal coloration Vessels of the pulp and also of the periosteum appeared normal with no suppuration

Legros and Magitot (42) who performed both clinical and experimental transplantations of teeth are best known for their latter work, which is now frequently referred to in many recent reports. In 1874 these investigators reported on the heterologous and homologous growth of transplanted tooth germs in dogs Their experiments are of interest since they were performed before aseptic precautions were advocated although their tissues were usually washed in solutions of bichloride of mercury That these investigators obtained growth and differentiation of transplanted tooth germs is important It is curious that under the conditions of their experiments they were unable to obtain satisfactory results when transplants were made between animals of the same zoological order although their transplants from newborn dogs to adult guinea pigs were successful Out of 88 attempts to transplant tooth germs, 10 were successful and did not succumb to resorption or suppuration. As a result Legros and Magitot felt that their experiments confirmed the work of Bert and others on grafts between animals of different zoological orders In this respect their work is considered important.

David in 1877 (11) in writing about animal experiments mentions Weisman who removed a dog's teeth and reinserted them immediately At the end of 3 days the animal was killed and after a fine injection had been given the vessels of the pulp and those about the root were examined. It was observed that the vessels in the reimplanted teeth were patent. One of these teeth was put on exhibition at the Bonn Museum.

In 1801 Fletcher (14) reported an experiment with a goat to which the teeth of a small cur had been transplanted.* In place of anesthesia a full meal was given before the operative procedure

* This experiment had first been attempted with a sheep that died as a result of other anesthesia

began. The dog's teeth were extracted, dried in a fire and the pulp chambers and root canals filled with gutta percha. Before transplanting, these teeth were soaked in a 1 1000 solution of bichloride of mercury and the operative site dusted with iodoform powder Originally transplants were made to sockets drilled in the cannon bone but later teeth from the dog were periodically transplanted to the host's jaws After a year the goat was killed and the teeth examined. Of 11 teeth transplanted only 2 underwent decalcification.

Delquel (1911) (43) reported on the grafting of teeth (44) He stated that transplanted teeth could not be called grafts because blood and nerve supplies were not maintained. He cited the work of Fredel, who experimented with 18 dogs and found that with extracted and reinserted teeth the pulp did not remain viable. These results do *not confirm the earlier work by Davkl.*

Huggin McCarrol and Dahlberg (1931) (33) attempted to bring about the formation of bone by the transfer of enamel epithelium to heterotopic locations They believed that tooth germ *elements should be capable of forming enamel and* dentin at various selected sites. They based their beliefs on the fact that teeth have been commonly found in teratomas and in other heterotopic locations such as the pituitary gland. Therefore they felt that teeth should be capable of development at selected sites outside the oral cavity They used unerupted permanent maxillary and mandibular canines of young dogs 3 to 6 weeks old. Autogenous transplants were made between the internal and external oblique muscles of the abdominal wall Various combinations of tooth germ elements were also transplanted and were allowed to remain at the transplantation site for varying periods up to 50 days. These authors had better success with autogenous transfers than did Legros and Magitot. The former believed that superior methods of maintaining sterility which had not been entirely understood during an earlier era contributed to their greater success

From their studies they observed that when the inner enamel *epithelium lost contact with the* odontoblasts the characteristic organization of the ameloblasts was lost There followed a dedifferentiation into stratified epithelium. In addition if this anatomic relationship was not maintained there was no enamel formation Degenerated enamel epithelium formed nests of epithelial cells and pearls but did not form cysts It is apparent from a comparison of results of various studies that the site of transfer has considerable bearing on the behavior of specialized or fated cells While some workers presented an opposite view Huggins and his colleagues questioned the influence of mesenchymal connective tissue derivatives on the form and function of epithelium. They observed that the structural characteristics of the odontoblasts were lost when this specialized mesodermal connective tissue was removed from the adjacent epithelium.

Willis (1935) (45) performed intracerebral homotransplants of minced embryonic tissues to white rats ranging in age from 2 weeks to 4 months Among the particular tissues used were minced upper and lower jaws The length of these donor embryos was about 1 6 cm. The transplanted tissues were allowed to remain in place from 4 to 8 weeks. Willis reported that growth of *the explants was "exceptionally vigorous* and felt that the brain was a highly favorable soil for implants. Despite the mincing of tissues the tooth germs transplanted escaped damage for Willis showed photomicrographs of fully devel*oped teeth.* With these experiments he found* that the age of embryonic tissues influenced the results since half grown embryos grew and developed more extensively than a nearly full term fetus.

Sutro and Pomerantz (1939) (46) transplanted tooth germ elements to marrow cavities of tibias in kittens The idea for this study arose from the occasional adamantinoma found in the tibia. The unerupted canines were exposed removed, transplanted and left in place from 3 to 180 days. These investigators found that there was considerable dentin formation and as they stated, "Differentiation seemed to be progressing. They also found that enamel and odontoblasts showed continued activity but when ameloblasts were absent transplanted odontoblasts did not show this continued activity nor did Hertwig's sheath survive and proliferate From their studies the authors concluded that ameloblastomas and adamantinomas were different.

*Hahn (1941) (31) using the unerupted maxillary* canines of 6-week-old female dogs autotransplanted various combinations of tooth germ elements to the ovaries. The transplants were

* If these tooth germs had been damaged during the mincing fully developed teeth would not have been recovered

allowed to remain in place from 7 to 40 days. Except for the transplantation site, this work follows a very similar pattern to that of Huggins and his associates. After 15 days, regular tubular dentin developed when pulp odontoblast tissue was transplanted Hahn found however that the presence of enamel epithelium was not essential for maintaining the form of dentin once deposits of the latter had begun and he further postulated that once ameloblasts began their enamel production they lost their organizing influence on mesenchymal tissue. In contrast to the earlier work of Huggins, McCarrol, and Dahlberg, there was a tendency for epithelial elements to form cysts in the ovarian tissue. Comparison of these two studies confirms the hypothesis that the ultimate fate of transplants differs according to the transplantation site.

Villafane and Gonzales (1942) (47) homotransplanted rat tooth germs to various sites,such as the marrow cavity of the tibia the spleen muscle and empty tooth sockets. The course of development prior to sacrificing the animals was followed by x-rays from the third to the one hundred eightieth day The authors reported that the extent of development was dependent upon the degree of continued relationship between enamel and dentin. Tooth germs transplanted to muscle did not grow a result differing from the findings of Huggins and his coworkers. The ameloblasts degenerated and only osseous formations of dentinoid or osteoid tissue were recovered. Tooth follicles inserted into empty alveolar sockets grew but remained small and histologic examination of recovered tissues revealed degenerated zones of ameloblasts. Transplants to the spleen did not show a normal morphologic development of the follicle but presented a picture of vacuolar degeneration which the authors claimed was due to a lack of nutritive elements necessary for the development of teeth Villafano and Gonzales compared tooth germs to glands and concluded that their experiments demonstrated a vital capacity of these explants to adapt themselves to heterotopic locations.*

Baker (1941) (48) as an orthodontist was interested in the influence of tooth development on jaw growth The rapid unequal growth of the skull during childhood and adolescence intrigued him. Baker therefore studied the influence of the formative organs of the teeth on the mandibles of fetal rats. The mandibles of unborn rats were transplanted to the leg muscles of the female parent three days prior to birth. This particular time was chosen because it was the peak of prenatal development, a rationale in contrast to the concept of Willis who believed earlier embryos attained better growth. The transplanted mandibles grew a third larger than the controls in 43 days, but they were smaller than the jaws of the litter members which were born. This experiment supported Dr E. A. Hooton's conviction that after a bone is shaped by inherent growth influences, development continues largely from functional muscle stimuli. Thus when an area of bone is not stimulated by muscular attachments, that particular part gradually resorbs.

Lapchinsky and Malinowsky (1940 1943) (49–52) attempted homeoplastic transplantation of the anlagen of teeth among cats rats and dogs. In three instances they transplanted to the femurs of adult rats, but generally they transplanted to curetted alveolar sockets following removal of the original teeth They also reported one unsuccessful transplant of scrapings of human tooth material to a dog. The aim of these Russian authors was to study the practicability of transplantation in human beings, but because of the small number of animals used and the paucity of successful takes little can be concluded from their experiments

Shapiro and MacLean (1945) (53) homotransplanted developing tooth germs to the mandibles of cats. Usually the donors were younger than the hosts, the former ranging in age from 4½ weeks to 5½ months and the latter from 1½ months to 1 year Sometimes donor and host were approximately of the same age. Developing tooth germs of canines or incisors were transplanted to either the permanent canine or premolar sockets. While these investigators worked with only a few animals it is of importance to note that they found that injured tooth germs resorbed when transplanted. They also observed no decrease in opacity of crowns completely formed before transplantation. Successful takes as observed by x-rays had a considerable increase in opacity by 5 weeks after the operation

Sato (1933) (54) in reviewing the work of Ogata (1920) noted that this investigator had observed that salivary glands have an endocrine as well as exocrine activity The endocrine activity of these glands appeared to cause a decrease

* These conclusions are not completely consistent with the findings of their experiments

in serum calcium, which was interpreted to be a result of transference of serum calcium to calcium reservoirs such as bone and teeth. In order to clarify the effect of the endocrine hormone on calcification of dentin studies were made of parotid gland extracts and transplants according to the method of S. Fuse. Rabbits were used and tooth germs were transplanted to the marrow cavities of tibia. Intravenous and subcutaneous injections of the extract were made into the host animal and the effect of this salivary gland extract upon calcification of the transplants was noted. The rate of dentin formation was determined by measuring what is called "the inter spaces" of dentin. It was observed that 15 mg. of the extract per kilogram of body weight at first caused a slowing and then an acceleration of dentin growth. It was also found that this parotid extract precipitable at a pH of 5.40 reacts qualitatively as well as quantitatively on the formation of dentin. Small doses accelerated dentin formation and medium doses inhibited it. Sato evaluated the effect of this hormone histochemically. His work implies that parotid gland extract as used in this instance has some effect on calcium and on phosphate mobilization.

This approach to problems of mineralization and transplantation could possibly be elaborated by using other transplantation sites where behavior of transfers could be more easily followed. In fact these observations call for intensive investigations in this area for it has been recently postulated that secretions of the salivary glands control the level of the thyroid hormone in the blood stream. This concept is related to degrading the iodide ion which is recycled to the thyroid. When the blood level of the thyroid hormone dropped in rats the incidence of caries was increased.




Fig. 177. Induction of tooth formation in the salamander. 1. Bilateral transplantation of neural crest beneath the abdominal epidermis showing the formation of optic (O), brain (B) and teeth (T). Section showing position of transplant. (Courtesy of Dr. James K. Avery, School of Dentistry, University of Michigan.)

Levy and Detwiler (1951) (55) and Avery 1950 1954) (56) have done experimental work with transplantation in salamanders (amblystoma punctatum or maculatum). Levy and Detwiler removed the mandibular arches from donor embryos and transplanted them to the region of the first to third somites in host embryos. The majority of transplants were successful. When oral epithelium was included in the graft formations of teeth were induced although new mandibular arches were not regenerated. Avery also extirpated selected areas of the neural crest at the time of neuralization and transplanted them homeoplastically to abdominal walls where teeth formed.* In addition the abdominal wall epidermis was induced by the underlying neural transplant to invaginate forming the enamel organ of the teeth. It was thus established by these investigators that certain neural crest cells in salamanders induce epithelium to organize and invaginate thus forming the anlagen of teeth.

Agnew and Fong (1956) (57) made histologic studies of autotransplanted teeth in monkeys. Developing third molars were transplanted in a manner similar to that recommended by Apfel. The report by these authors present an interpretation of histologic findings at intervals from 4 days to 2 years. Success of these experiments depends on development of a healthy periodontal membrane and the maintenance of a normal cellular pulp.† As root formation progresses irregu-

* Tissues were selected at stage 15 (rotation stage) and included anlagen of the eye and brain. Thus these structures developed in a normal relationship with the teeth.

† These observations support the view of many clinicians.

larly deposited osteodentin becomes covered with tubular dentin. This regeneration or replacement seems dependent upon survival of odontoblasts in vital pulp areas. It is also important for the success of transplantation that teeth have partially developed roots with wide apical openings.

### Experimental Work by Fleming

The author (1952–1958) (58–72) successfully transplanted tooth germs into the anterior eye chambers, the brains and the axillae of various laboratory animals (60–72). These transplants were made both homeoplastically and heteroplastically. Tooth germs obtained from human embryos and fetuses were also transplanted to experimental animals (67). These studies showed that tooth germs so transplanted had the ability to grow and develop and eventually form mature tooth structures. In addition, a histopathologic study was made of the effect of carcinogenic chemicals and fluorides on the growth and development of tooth germs transplanted to the above sites (60, 62, 65, 70). It is interesting to note that recent experiments with transplanted tooth germs and the Shope papilloma virus raise particular question about the biologic behavior of different cell layers associated with developing teeth (72). Homotransplants of tooth germs to female guinea pig castrates attained a greater size than was previously obtained in any of these experiments (68). Subsequently it was likewise observed that homotransplants of tooth germs in guinea pigs also attained a greater than expected size when transplants were made simultaneously with a human glioblastoma multiforme (71). Results are presented and discussed on the following pages, accompanied by general comments on tissue transplantation.

"Why transplant tooth germs? What can be gained by experimental transplantation?" Dr Hamilton B. G. Robinson recently commented. "Fleming's ingenious method of growing tooth germs outside the oral cavity offers another approach to the study of the normal development of teeth and to pathologic disturbances in growth."

Investigators at first examined the behavior of a number of tissues at selected transplantation sites with the aim of studying the biologic laws of transplantation. Thus, they originally concerned themselves with the status of tissues following transfer. The reactions of adult and embryonic normal tissues and of benign and malignant




FIG 128 Tooth germ transplants *1* Eye of a guinea pig bearing a transplant of a guinea pig tooth germ for 14 days *2* Eye of a guinea pig bearing a transplant of a human tooth germ for 20 days *3* Eye of a guinea pig bearing a transplant of a dba mouse tooth germ for 23 days *4* Eye of a guinea pig bearing a transplant of a human tooth germ for 40 days *5* Axilla of a dba mouse showing a homotransplant of a dibenzanthracene treated 15-day tooth germ after 104 days (*1* and *2* reprinted with permission of the editors of the Journal of Dental Research)

neoplasms have been extensively examined at various sites. Interests have progressively broadened and various experimental procedures have been applied to influence subsequent growth and development of these tissue transplants. This extension of ideas and an improvement in histologic techniques have resulted in a better understanding of factors involved in survival, growth and development of transplants at specific locations within the host animals. While it is evident that much progress has been made many problems remain to be solved—some relating to persistence as well as to growth and development of transplanted tissues.

Survival of transplants depends upon acceptance by the hosts. When tissues are transplanted, they must initially go through a latent period which lasts 24 to 48 hours. Following this there is a reaction or response by the host which is manifested in part by an accumulation of small round

in serum calcium, which was interpreted to be a result of transference of serum calcium to calcium reservoirs such as bone and teeth In order to clarify the effect of the endocrine hormone on calcification of dentin, studies were made of parotid gland extracts and transplants according to the method of S Fuse. Rabbits were used and tooth germs were transplanted to the marrow cavities of tibias Intravenous and subcutaneous injections of the extract were made into the host animal and the effect of this salivary gland extract upon calcification of the transplants was noted The rate of dentin formation was determined by measuring what is called "the inter spaces" of dentin. It was observed that 15 mg. of the extract per kilogram of body weight at first caused a slowing and then an acceleration of dentin growth It was also found that this parotid extract precipitable at a pH of 5.40 reacts qualitatively as well as quantitatively on the formation of dentin Small doses accelerated dentin formation and medium doses inhibited it. Sato evaluated the effect of this hormone histochemically His work implies that parotid gland extract as used in this instance has some effect on calcium and on phosphate mobilization.

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Fleming's ingenious method of growing tooth germs outside the oral cavity offers another approach to the study of the normal development of teeth and to pathologic disturbances in growth.

Investigators at first examined the behavior of a number of tissues at selected transplantation sites with the aim of studying the biologic laws of transplantation. Thus, they originally concerned themselves with the status of tissues following transfer The reactions of adult and embryonic normal tissues and of benign and malignant neoplasms have been extensively examined at various sites Interests have progressively broadened and various experimental procedures have been applied to influence subsequent growth and development of these tissue transplants. This extension of ideas and an improvement in histologic techniques have resulted in a better understanding of factors involved in survival growth and development of transplants at specific locations within the host animals While it is evident that much progress has been made many problems remain to be solved—some relating to persistence as well as to growth and development of transplanted tissues.

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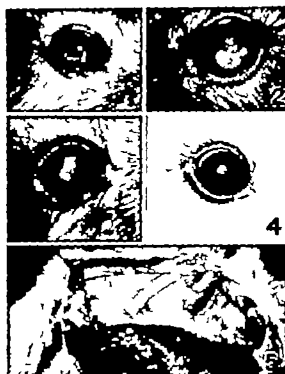



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cells or lymphocytes about the transplants If the transfers are not destroyed by this lymphocytic attack they ordinarily become stromatized and vascularized. This response on the part of the host which is not clearly understood, may vary in intensity and duration according to the tissues transplanted, the sites used and the type of recipient. In certain experiments x-rays and/or cortisone have been used to suppress the mobilization and number of circulating lymphocytes thus conditioning the hosts for acceptance of transplants and enhancing their subsequent growth. While growth of embryonic tissues in *the author's laboratory was not improved by the* use of x-rays and cortisone other investigators obtained favorable results with transplanted malignant neoplasms.

Most embryonic and a number of neoplastic tissues have a common autonomous property that allows them to survive homologous as well as heterologus transplantation. It was demonstrated that adult tissues generally are capable of surviving and growing upon autologous transfer and survive but do not grow upon homologous transfer These adult tissues, however have not yet demonstrated consistent survival of heterologous transfer under transplantation methods ordinarily employed. In the latter instance an intense lymphocytic reaction takes place. It is generally accepted that normal adult tissues have *inherent properties which are not present in* either embryonic tissues or certain malignant neoplasms and as a result these adult tissues upon transplantation elicit a different response from the host.

It has been the experience of a number of investigators that in the anterior eye chambers and brains the lymphocytic response is less vigorous. Thus, in this respect these locations are better suited for the transplantation of embryonic tissues such as tooth germs According to some investigators who transplanted tooth germs to other than the above locations, there was usually a *rapid resorption of the tissues.*

The age of embryonic tissues is an important factor in relation to their growth when transplanted. Tooth germs at an early stage of development best survive *in vivo* transplantation. In general, transplantation of tooth germs in the author's laboratory was most successful when embryonic tissues prior to calcification were used. When well calcified tooth structures were transplanted there was usually a vigorous foreign *body reaction which ultimately led to resorption.* Transplantation of slightly calcified tissues caused a moderate inflammatory reaction but in many instances the transplants survived

Mouse tooth germs from 14 to 16-day-old embryos guinea pig tooth germs from 25 to 35-day-old embryos and rabbit tooth germs from 21 to 25-day-old embryos were best suited for transfer

The best results were obtained with viable non-contaminated non-calcified whole embryonic tooth tissues Once these transplants became stromatized and vascularized, growth, development, and differentiation were usually continuous and rapid with a greater rate of development obtaining in the eyes and brains than in the axillae When tooth germs were transplanted to the anterior eye chambers *growth was usually confined* to this area This was due to intraocular pressure and mechanical conditions which had a limiting effect on their ultimate size Moreover microscopic study proved that the pulps and supporting tissues were more compact in the anterior eye chambers than in either the brain or axillae. It was also evident that these embryonic tissue transplants were subject to different fates for identical tissues transplanted under like conditions and to litter mates showed variations in growth, development and maturation. Recently in the author's laboratory it has been *possible under specific conditions to enhance the* growth of tooth germs in the eye so that these tissues overcame intraocular pressure and mechanical restrictions and thus ruptured the cornea. Such exuberant growth and development took place when tooth germs were transplanted to the eyes and brain of female guinea pig castrates or to the eyes of guinea pigs when the embryonic teeth were transplanted at the same time and to the same location with a human brain tumor (68 71)

Transplantations of tooth germs were successfully carried out between animals of the same and *different strains and from man to lower animals* (58 67) Whole or parts of tooth germs retained their ability for growth, development and differentiation when removed from embryos or fetuses and transplanted to the eyes brain, or axillae of experimental animals In mice growth in the axillae and brains was much superior to intraocular growth. In guinea pigs intracranial growth was equal and in certain respects superior to intraocular growth With *human transfers the best*

growth and development were obtained in the anterior eye chambers of guinea pigs. In rabbits homologous eye transfers were extremely satisfactory.

In these experiments normal relationships of the internal epithelial layer of the enamel organ and of the mesenchymal layer of the dental papillae were not always maintained during or following transplantation. Despite these mechanical factors, it appeared that ameloblasts, when transplanted without adjacent tissues often continued differentiation and at times formed enamel. It should be noted, however that the rate of enamel formation in these particular instances was comparatively slow.

The germinal layers formed in the embryo give rise to tissues subject to a law of specificity. As development progresses and tissues differentiate, each emerging tissue is particularly bound to a less specialized parent tissue. From the parent tissue, the oral epithelium, there is development or emanation of specialized tissues and as a result certain cells of the tooth germ arise. These cells are primarily those of the inner and outer enamel epithelium. After development of the cells of the inner enamel epithelium, the ameloblasts undergo certain chemical and histologic changes to become functional and as a result lose their capacity to continue mitotic division. The functional status or activity of these cells is indicated by their length and also by the polarity of their nuclei. Those that will form enamel rods elongate; those that will not remain short.

At the height of this metabolic activity the demand for nourishment is very great, exceeding that supplied from adjacent tissues and at particular times taking precedence over the needs of other organs. Eventually materials necessary for the formation of the enamel matrix are drawn directly from the bloodstream. Therefore, an insufficient amount of metabolites available from the blood may be responsible for a lack of enamel formation and for degeneration of these highly specialized cells.

Variations in the growth and differentiation of ameloblasts, which may have been due to several factors were observed in the present study. In these experiments ameloblasts either functioned normally or as dedifferentiated cells and never formed a neoplastic growth such as an ameloblastoma. On failure to form enamel ameloblasts lost their tall columnar structure and sometimes clusters or cords of epithelial cells resulted. These seemed to arise from the junction of the outer and inner enamel epithelial layers. At other times epithelial pearls or keratinized material was formed. In experiments carried over long periods these keratinous areas degenerated into cysts. At other times the nuclei of the ameloblasts became pyknotic, their cell walls ruptured and their cytoplasm was lost. It should be stressed, however that under limits of these experiments ameloblasts usually laid down the pre-enamel matrix which later formed mature enamel. The expected behavior of the ameloblasts, as will be enlarged upon later was altered under certain conditions.

Once the cells of the subjacent mesenchyme became differentiated, odontoblasts were formed and acquired a specific potentiality for participation in dentin formation. When a normal relationship was lost between the ameloblasts and odontoblasts, independent dentin formation continued either in a recognizable tubular pattern or in islands. Once dentin formed in these tooth germ transplants, the blood supply to the pulpal areas gradually decreased and there was a slow but constant reconstitution of dentin (63 66). Collagen fibers became more evident, forming bridge-like patterns along the inner border of the dentin. There followed an obliteration of pulpal areas by an osteoid tissue.

This change within the pulpal areas of transplants and the revision of dentin took place earlier and at a faster rate when the morphology of the tooth germs had been lost during transfer. Under certain conditions (to be enlarged upon later) cartilaginous rather than osteoid structures appeared in association with the dentin of the transplants and within the dental pulps. As these results are interpreted additional information regarding the comparative crystalline organization and structural density of enamel, dentin and bone may be obtained. Enamel, dentin and bone all have the same molecular structure of hydroxyapatite, with enamel having the most densely packed molecular form and bone the least. The presence of cartilaginous structures may be an indication of an inability of these pulpal areas to revise themselves into osteoid tissues.

It has been possible to retard maturation and calcification of tooth germs by conditioning the hosts so that there was a resultant effect on the transplants. This was accomplished by adding certain compounds such as fluorides, to the




FIG 120 Tooth germ transplants *1* Homotransplant of a tooth germ from a 20-day guinea pig embryo after 80 days in the brain ×208 *2* Homotransplant of a tooth germ from a 21-day guinea pig embryo after 46 days in the anterior chamber of the eye ×44 *3* Homotransplant of a tooth germ from a 23-day guinea pig embryo after 30 days in the brain Host had received 17 injections of sodium fluoride (1 1,000) ×60 *4* Homotransplant of a tooth germ from a 23-day embryo after 5_ days in the brain of a guinea pig Host received oral sodium fluoride (1 75,000) ×60

*a* ameloblast *br* brain *c* cartilage *d* dentin *e* enamel *i* iri *od* osteodentin *p* pulp (*1 2* and *3* reprinted by permission of the editors of the Journal of Dental Research )

drinking water of animals bearing transplants or by injecting equivalent amounts of these compound

Retardation in the calcification of transplants was observed when fluorides in concentrations of 1o to 20 parts per million were added to the drinking water or equivalent amount in micrograms were injected intraperitoneally (62) While the calcification was retarded in transplant in fluoride treated animal there was a thicker amount of pre-enamel and predentin laid down significantly in untreated control animals

the rate of calcification was more rapid. In fluoride treated animals for the periods observed, which were up to 90 days, there was a consistent retardation of calcification. Investigators have shown that with certain fluoride compounds, such as sodium fluoride, there is an inhibitory action with phosphorylating mechanisms. One explanation attributes this action to the $F^-$ ion. It has also been shown that phosphorylating mechanisms are concerned with enzyme systems responsible for transport of metabolites across cell walls.

While there is no evidence to show that the $F^-$ ion passed the cell membrane and was active within the cytoplasm or nucleus, the presumed activity at the cell wall is sufficient to account for the phenomena observed. With the compounds sodium fluoride and calcium floride similar results were obtained with concentrations diluted down to 1:75,000.

Phosphorylating mechanisms are an important part of energy production and where they are inhibited energy production may be retarded. It has been shown that during mineralization and calcification of teeth, cells actively participating in this metabolic process at specific times requires vast amounts of energy. From the author's studies it would appear that the inhibitory action of the $F^-$ ion is manifested most as pre-enamel and predentin are being laid down, at which time enormous amounts of energy are in demand. These alterations in calcification are probably due to the blocking of carbohydrate utilization by tying up enzyme systems concerned with phosphate transport, which in turn supply the energy for the mobilization of calcium. Thus, the $F^-$ ion is only indirectly associated with calcification.

When fluorides were administered in addition to the retardation of calcification there was an alteration in the cuticular structure of ameloblasts. This caused them to appear thicker which may have been due to an affinity of the fluorides for the scleroproteins forming the walls of these cells. Keratin is the end product of the activity of cells of both the inner and outer enamel epithelium. Ordinarily keratin formations were abundant when the tissues had been treated with chemical carcinogens such as methylcholanthrene or dibenzanthracene before transplantation. However in the presence of fluorides there was a retardation of keratin formation in both normal and methylcholanthrene treated transplants.

This lack of keratin formation in direct association with transplanted tooth germs in fluoride treated animals was most significant. It has previously been mentioned that dentin ordinarily underwent a metaplasia or reversion into an osteoid tissue. When the host animals were given fluorides no such changes took place; instead, cartilaginous tissue was frequently found in pulpal areas in close association with dentin. It was further observed that when fluorides were given to the hosts, there was an alteration in the walls of blood vessels supplying the transplants. In addition there appeared to be an increased lymphocytic response which was manifested by focal accumulations of small round cells about the transplants and peripheral cuffing of these cells around blood vessels or capillaries adjacent to these transfers. Such factors may have been responsible for the retarded development and maturation of tooth germ transplants under fluoride administration.

It has been established that certain polycyclic hydrocarbons can evoke malignant changes when brought in contact with living tissues. At first, attention was focused upon their tumor producing properties, but it was later observed that these compounds were also capable of inhibiting growth of malignant tumors. In the author's laboratory certain carcinogenic compounds have been routinely transplanted with embryonic tissues and for several years their effect on transplanted tooth germs has been studied (60, 65, 70).

When the carcinogenic chemicals 20-methylcholanthrene and 1,2,5,6-dibenzanthracene, were synchronously transplanted with tooth germs both growth and development were affected in several ways. The results with each of these chemical compounds were substantially similar except that dibenzanthracene occasionally exhibited an early toxic effect in animals bearing brain transplants. In the presence of these carcinogens tooth germs usually became calcified within 18 to 20 days following transplantation. By 30 days a proliferation of cells away from the main body of the transplant began. Adjacent to the transplants accumulations of lightly eosin stained cells, presumably from the outer enamel epithelium were observed. Keratin formations now became evident. Cystic areas began to make their appearance by 40 days and seemed to




Fig. 129. Tooth germ transplant. *1*. Homotransplant of a tooth germ from a 20-day guinea pig embryo after 80 days in the brain. ×208. *2*. Homotransplant of a tooth germ from a 23-day guinea pig embryo after 16 days in the anterior chamber of the eye. ×44. *3*. Homotransplant of a tooth germ from a 23-day guinea pig embryo after 30 days in the brain. Host had received 1[illegible] injections of sodium fluoride (1:1,000). ×60. *4*. Homotransplant of a tooth germ from a 23-day embryo after 5[illegible] days in the brain of a guinea pig. Host received oral sodium fluoride (1:75,000). ×60.

*a*, ameloblast; *b*, brain; [illegible] cartilage; *d*, dentin; *e*, enamel; *c*, ...; *od*, osteodentin; *p*, pulp. (*1*, *2* and *3* reprinted by permission of the editors of the Journal of Dental Research.)

drinking water of animals bearing transplants or by injecting equivalent amounts of these compounds.

Retardation in the calcification of transplants was observed when fluorides in concentration of 1[illegible] to 20 parts per million were added to the drinking water or equivalent amounts in micrograms were injected intraperitoneally (6[illegible]). While the calcification was retarded in transplants in fluoride treated animals there was a thicker amount of pre-enamel and predentin laid down. Significantly in untreated control animals

the rate of calcification was more rapid. In fluoride treated animals for the periods observed which were up to 90 days, there was a consistent retardation of calcification. Investigators have shown that with certain fluoride compounds, such as sodium fluoride, there is an inhibitory action with phosphorylating mechanisms. One explanation attributes this action to the $F^-$ ion. It has also been shown that phosphorylating mechanisms are concerned with enzyme systems responsible for transport of metabolites across cell walls.

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It has been established that certain polycyclic hydrocarbons can evoke malignant changes when brought in contact with living tissues. At first, attention was focused upon their tumor producing properties but it was later observed that these compounds were also capable of inhibiting growth of malignant tumors. In the author's laboratory certain carcinogenic compounds have been routinely transplanted with embryonic tissues, and for several years their effect on transplanted tooth germs has been studied (60, 65, 70)

When the carcinogenic chemicals 20-methyl cholanthrene and 1 2 5 6-dibenzanthracene were synchronously transplanted with tooth germs, both growth and development were affected in several ways. The results with each of these chemical compounds were substantially similar except that dibenzanthracene occasionally exhibited an early toxic effect in animals bearing brain transplants. In the presence of these carcinogens tooth germs usually became calcified within 18 to 20 days following transplantation. By 30 days a proliferation of cells away from the main body of the transplant began. Adjacent to the transplants accumulations of lightly eosin stained cells, presumably from the outer enamel epithelium were observed. Keratin formations now became evident Cystic areas began to make their appearance by 40 days and seemed to




FIG 129 Tooth germ transplant *1* Homotransplant of a tooth germ from a 20 day guinea pig embryo after 80 days in the brain ×205 *2* Homotransplant of a tooth germ from a 23-day guinea pig embryo after 40 days in the anterior chamber of the eye ×44 *3* Homotransplant of a tooth germ from a 23 day guinea pig embryo after 30 days in the brain Host had received 17 injections of sodium fluoride (1 1,000) ×60 *4* Homotransplant of a tooth germ from a 23-day embryo after 52 days in the brain of a guinea pig Host received oral sodium fluoride (1 5,000) ×60

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drinking water of animals bearing transplants or by injecting equivalent amounts of these compounds

Retardation in the calcification of transplant was observed when fluorides in concentrations of 1.5 to 20 parts per million were added to the drinking water or equivalent amount in micrograms were injected intraperitoneally (62) While the calcification was retarded in transplant in fluoride treated animals there was a thicker amount of pre-enamel and predentin laid down significantly in untreated control animal

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FIG. 130. Dibenzanthracene treated tooth germs. *1.* Homotransplant of a dibenzanthracene treated tooth germ from a 25-day embryo recovered after 45 days in the brain of a guinea pig. Note occluded blood vessels and hemorrhagic pulpal area. ×45. *2.* Homotransplant of a dibenzanthracene treated incisor from a 25-day embryo recovered after 150 days in the brain of a guinea pig. Note keratinous areas, cyst, epithelial pearl, and epithelial nests of cell. ×24.

*br* brain, *b.v.* blood vessels, *cy* cyst, *d* dentin, *e* enamel, *e.n.* epithelial nests of cell, *e.p.* epithelial pearl, *h.a.* hemorrhagic area, *k* keratin, *p* pulp.

coalesce, thus forming larger areas. By 70 to 80 days keratin formations stained much more heavily and were frequently observed arising from the periphery of groups of epithelial cells.

In transplants that had been in place with carcinogens for the longer period, it was necessary to stain for sulfhydryl groups to differentiate keratin from collagen. The Barrnett-Seligman test for protein bound or fixed sulfhydryl groups was employed, and the positive reaction obtained for sulfhydryl groups indicated that tissues which had been designated as keratin were keratin and not collagen. Multicystic areas were observed in many places in transplants surviving for longer periods, and islands or groups of epithelial cells lay in a disorderly fashion close to tooth formations. Some of these nests of cells assumed a palisaded arrangement tending to break down in the center as new cells formed about the periphery. This occurred more frequently after 120 days. Older keratin formations in some areas now appeared to be losing their form and were more frequently amorphous, but new formations also seemed to arise from groups of epithelial cells. For these longer periods calcification was poor, dentin often being associated with cartilaginous structures. From 120 days to about one year, the maximum length of time of these experiments, a moderate amount of hyperplasia of epithelial cells showing pleomorphism and hyperchromaticity was evident. Epithelial cells that met some of the criteria of malignancy were probably the persisting cells of the outer enamel epithelium. The greatest effect of these chemical carcinogens was on the epithelial and not on the mesenchymal cells. In studies completed with the Shope papilloma virus it was found that there was a similarity of the keratin formed when methylcholanthrene or dibenzanthracene was applied to the tooth germs before transplantation (72).

In the transplantation of human tooth germs to lower animals the most favorable site was the anterior eye chambers of guinea pigs and the axillae of dba Bar Harbor mice. Transplants of human tooth germs to these locations showed growth and development with formation of mature tooth structures. The best results were obtained with tissues from 16- to 18-week-old fetuses. These transplants survived and maintained themselves, producing calcified enamel and dentin. Tooth germs from embryos ranging in age from 8 to 12 weeks grew more slowly and did not increase much in size, although recovered tissues were found to be small duplications of mature teeth. Tooth germs or developing teeth from older fetuses were too large to be transplanted successfully to the location selected. Although parts of them sometimes survived, these large transplants generally degenerated, and recovered tissues from the transplantation areas failed to show remains of the transplant.

The major problem in the transplantation of human tooth germs has been in obtaining viable

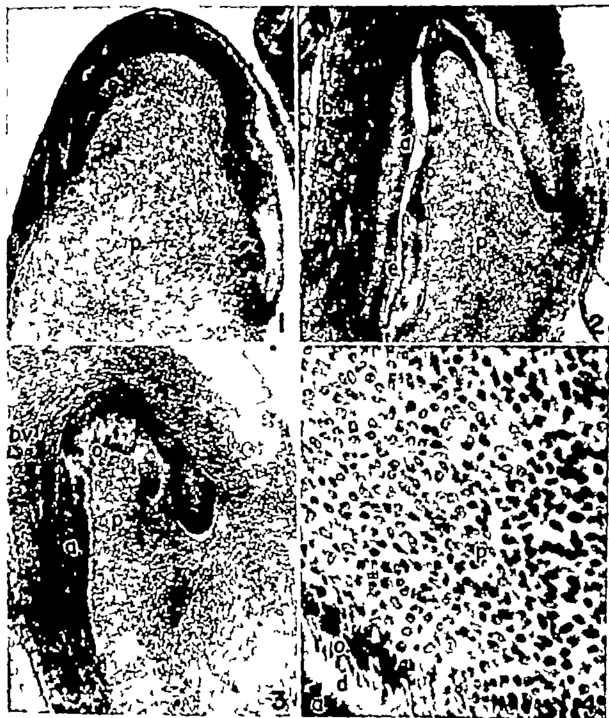



FIG 131 Human tooth germ transplants *1* Section showing transplant of a tooth germ from a 4 month human fetus after 62 days in the anterior eye chamber of a guinea pig Original magnification ×40 *2* Section of a lower second incisor from a 4 month human fetus after 90 days in the anterior eye chamber of a guinea pig Original magnification ×165 *3* Section showing transplant of a tooth germ from a 4-month human fetus after 30 days in the anterior eye chamber of a guinea pig Original magnification ×145 *4* Higher power of pulpal area in *3* to show mitotic figures Original magnification ×450

*a* ameloblasts *bv* blood vessels *d* dentin *e* enamel *i* iris *o* odontoblasts *p* pulp (*1* and *2* reprinted by permission of the editors of the Journal of Dental Research )

non-contaminated human material of a satisfactory age and condition. The suitability of human tissues used could not be determined prior to transplantation, and occasionally what appeared to be very desirable tissue failed to grow The author's laboratory is attempting to solve the problem of the storage of human tooth germs and to ascertain the conditions

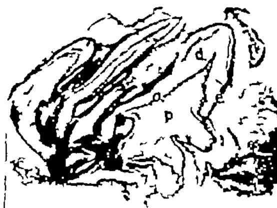



FIG 132 *Homotransplant of guinea pig molar* after 90 days in the anterior chamber of the eye Tissue for transplant was obtained from a 30-day embryo Original magnification ×30

*d* dentin *e* enamel *i* iris *o* odontoblast *p* pulp

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In the preparation of this chapter over 300 articles relating to the clinical transplantation of teeth were reviewed The majority of these were case reports of a similar nature of which a representative group were chosen for inclusion in the clinical review In the review of the *in vitro* and *in vivo* studies the entries included represent the most significant work in these area

The author wishes to acknowledge the aid of Mrs Rita Fernson in the preparation of this chapter


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Fig 132 Homotransplant of guinea pig molar after 20 days in the anterior chamber of the eye Tissue for transplant was obtained from a 30-day embryo Original magnification ×30

*d* dentin *e* enamel *i* iris *o* odontoblasts *p* pulp

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CHAPTER 10

# *Transplantation of Teeth in Humans*

MIKLOS CSEREPFALVI, M.D.

## Historical Background

Transplantation of teeth in humans preceded animal experiments in this field by several centuries. Nevertheless, current experiments relating to human transplantation are based to a large degree on animal experiments. Archeologic findings prove that the problem of dental replacement has engaged the attention of mankind since ancient times. Relics from excavations in Egypt show the results of primitive attempts to replace teeth. Ancient methods of dental replacement were largely empirical but they were of scientific value because they turned the attention of research workers in this direction.

In the literature on dentistry we find the first reference to the transplantation of teeth in the ancient writings of Chinese, Indian and Arabic physicians. Detailed data were published in Fouchard's book, Le Chirurgien Dentiste, in 1780 (1). He described some successful transplantation of teeth performed by Ambrose Paré, who lived in France in the sixteenth century and who acquired great fame by practicing the transplantation of teeth. Pfaff (2) in 1755 made a critical analysis of his own experiments and of those of his colleagues. His writings are too subjective, however, and contain little objective and scientific proof of his result.

Hunter (3) in 1771, 1777, 1780 and 1786 wrote articles in which he described animal experiments with dental transplants. In one of his experiments he observed the survival of a tooth that had been implanted into a rooster's comb. Philippeaux (4) conducted similar interesting experiments on the transplantation of teeth at about the same time as Hunter was similarly engaged. The scientific debate that followed revolved around the organization of the transplanted tooth. The purpose of transplanting a tooth into the rooster's comb is not known. Possibly this was done to store the tooth. It may be assumed that these were the first attempts to put away teeth for future use.

On the basis of the experiments carried out by Hunter and Philippeaux, another worker by the name of Wooffendalle (5) performed some experiments of his own relative to dental transplantation and published his findings in 1783. Further scientific debate ensued, and Palmer (6), Vasey (7) and Serre (8) expressed their doubts about the practical value of dental transplantation. Later, in 1807, Schmidt, in his monograph entitled *"Theorie und Erfahrung über die Zähne"* expressed a similar uncertainty regarding the application of tooth transplants to humans. Others, namely Richerand, Zang (9) and Mitscherlich (10) were even more emphatic in seeing no practical value in the transplantation of teeth. Indeed, some regarded this practice as an antisocial and barbaric operation. This opinion was based largely on the circumstances under which the experiments were carried out. For instance, if because of unbearable toothache it was necessary to extract an eyetooth from a high ranking officer, a soldier whose eyetooth met the requirements of the officer in respect to size and color was selected to provide the replacement. His tooth was removed and successfully transplanted to the jaw of the officer. In another instance a lady of social standing had the front teeth of her female servant extracted to replace her own. In the Middle Ages it is known that

dentists obtained teeth by extracting them from indigent and derelict subjects and transplanted them to the mouths of their well-to-do patients.

Younger (11) continued his experiments during the era of debate and in 1886 he reported the successful outcome of an operation in which a tooth was transplanted into an artificially cerated alveolus. Patterson (12) was concerned with the problem of dental reimplantation Bugnot (13) in an article dealing with the successful results in several cases recommended transplantation of embryonal tooth buds. Thus, his report may be considered the first one to contain the idea of transplanting tooth buds. He was also a pioneer in advancing the concept of heterotransplantation. Hipple (14) however was the first investigator who tried to appraise the subject of dental transplantation scientifically In 1890 he emphasized that the histologic and roentgenologic examination of the results were just as important as the operative technique Fletcher (15) in 1891 intensively investigated the role of the periodontium in the transplantation of teeth. This study was prompted by the observation of absorption in the unsuccessful transplantations

During this period of doubt and debate regarding the usefulness of dental transplantation there were investigators who began to experiment with implantation of foreign body materials. Znamesky in 1891 reported his successful results with the implantation of artificial teeth and the *adherents of his method disagreed only* in the choice of the composition of such artificial teeth. The results of later experiments and the pertinent literature were not widely noted in those days. But contemporary literature contains detailed data concerning similar experiments. Berner (16) in 1943 Weinberg (17) in 1950 Strain (18) in 1954 and a number of European workers report seemingly successful results. These experiments generally consist of placing and fastening in the alveoli or subperiosteally artificial teeth made of porcelain, vitallium or acrylic resin.

In this writer's opinion the experiments with artificial implants are certain to lead to failure because they are contrary to the biologic principle that no living tissue can survive without ill effect any long lasting pressure exceeding the capillary pressure

Recent investigators of human dental transplantation borrowed heavily from the literature concerning experiments with animals. Many excellent workers have been carrying out animal experiments. Huggins (19) in 1934 reporting his experiments on dogs included many of their data Later investigators include Sutro (20) in 1939 (on cats), Hahn (21) in 1941 (on dogs) Willfano (22) in 1942 (on rats) Lapchinsky (23) in 1940 (on cats and dogs) Shapiro (24) in 1945 (on cats) Sato (25) in 1953 (on rabbits) Avery (26) in 1950 (on the salamander) Agnew (27) in 1955 (on the monkey) Fleming (28) in 1952 to 1956 (on guinea pigs)

## CRITICAL ANALYSIS OF EXPERIMENTS IN THE PAST

While the archeologic findings of dental implants are interesting and amazing they have no scientific value. Prior to the eighteenth century the transplantation of teeth was practiced mostly by barbers and similar artisans, who performed these feats for purposes akin to showmanship

In the eighteenth century some scientific papers on the transplantation of teeth were published and these can be evaluated. It is certain that many experiments with implants of teeth were unsuccessful. This is borne out by the criticism of some authors who wrote about the unpleasant sequelae of these experiments.

Bugnot (13) expressed the opinion that results *would be much better if embryonal tooth buds* could be used instead of fully developed teeth. Apparently he disregarded the fact that it requires six to eight years under optimal conditions until an embryonal tooth bud develops to a normal tooth. Bugnot's idea had few adherents.

The work of Palmer and Vasey led to a significant scientific debate inasmuch as they investigated the role of dental tissue such as periodontium, cementum and pulp in the transplantation of teeth They also called attention to the danger of inoculation with some infectious disease (such as syphilis) The lively debate in the literature of that particular period reveals that the results which were claimed at first were not substantiated At that time the best results were obtained by reimplantation of teeth

Though the practical value of reimplantation of teeth may be debatable, the favorable results recorded are a sound proof of the feasibility of biologic replacement of teeth

All discussions in the literature were concerned mainly with the adequate preparation of teeth before retransplantation and also with the advisability of removing or retaining the periodontium. The operative techniques described correspond to the level of medical knowledge current at that time. The prevailing opinion of the period estimated the life expectancy of a reimplanted tooth as four to five years.

Even though these earlier successes could not bring about a general acceptance of reimplantation of teeth in man, we find a few enthusiastic advocates.

In the more recent literature Wilkinson (29) in 1926 and Axhausen (30) in 1936 reported their pathologic and histologic observations. Permt's publication (31) in 1948 reflect considerable clinical experience in this field and even mention cases in which teeth survived for ten years following reimplantation.

Miller (32) in 1956 refers to several cases where teeth that were lost through accident were successfully reimplanted. His experiments show that in teeth of young individuals in whom root development is still in progress, the pulp regenerates after reimplantation, while in fully developed teeth the roots must be prepared before the procedure is carried out. Miller mentions that in one instance a tooth remained alive eleven years after reimplantation.

Perry (33) in 1956 described the successful reimplantation of two incisors following an accident. Olech discussed similar successful implants. Emmertsen (34) in 1956 presented several cases of successful reimplantations in patients ten to sixty years of age. An increasing number of reports are appearing in which successful tooth reimplantations are recorded, especially following accidents.

## AUTOTRANSPLANTATION OF TEETH

Autotransplantation plays a very minor role in the problem of implantation of teeth. In actual practice it amounts to transplanting a third molar to replace a missing first molar. Obviously the essential step is to find a viable tooth in a developmental sac (20) and transplant it. Thoma (35) and Keresztesi (36) described such cases.

Agnew (27) (1956) discussed several successful autotransplantations of third molars accompanied by a thorough and detailed histologic study of the transplanted teeth. Hale (37), Apfel (38) and Thoma (35) refer to similar successful autotransplants of third molars.

From personal experience the author is inclined to believe that autotransplantation of third molars does not have very much practical significance simply because the removal of such teeth without much trauma to the teeth or to other oral tissues is difficult. The injured tooth may become too weak to survive in its new surroundings, or these surroundings may be entirely too crowded to receive it. All too often one may find at the end of a harrowing difficult procedure that the tooth is injured and incapable of surviving transplantation. Such an outstanding researcher as Hale (37), however, reported good results with autotransplantation in many cases.

## HOMOTRANSPLANTATION OF TEETH

Homotransplantation of teeth obviously would have a tremendous significance in dentistry, but there are several problems that must be solved before it becomes a practical possibility.

Since the author found no mention in the recent literature of systematic experiments with homotransplantation of teeth in humans, his own experiments in this new field are herein described.

The first experience was gained in 1935. A 7-year-old boy was brought to the Dental Division of the Kassab Polyclinic in Budapest, Hungary, because the dentist in attendance had decided that the boy's condition of extreme restlessness was due to a deciduous tooth, which he proceeded to extract. During the extraction he removed not only the involved deciduous tooth but also the permanent one located beneath it. When consulted about his problem by the dentist, the author replaced the bud of the permanent tooth and sutured the overlying gum. Two months later the replaced tooth began to appear through the gum and during the next three years it developed into a fully usable tooth (although somewhat rotated along its longitudinal axis).

In 1953 it became possible for the author to undertake the investigation of tooth germ transplants, experimenting with auto-, homo- and heterotransplants of teeth in dogs. The results of these animal experiments led the author to make his first homotransplantation of human teeth on February 13, 1954, when a canine tooth from a 10-year-old boy was implanted into a 45-year-old

woman. The successful outcome of this experiment led to the formulation of plans for a series of systematic experiments directed towards the solution of various problems connected with homotransplantation of teeth. These problems include 1) investigation of serologic and immunobiologic reactions between the transplant and the host 2) investigation to determine the stage of development of the tooth bed that is most favorable for a transplant 3) comparison of the results of transplantation with fresh and stored buds 4) examination of the development of implanted buds in tissues such as muscle bone skin and others 5) study of factors which influence the survival and continued development of transplants, and 6) investigation of the viability and transplantability of buds removed at various intervals after death (preferably accidental)

Practical considerations are to find ways and means of establishing "tooth banks" and to perfect the surgical technique of transplantation

In the initial experiments with fresh transplants the author used the following methods autotransplantation (supernumerary teeth and third molars) in young subjects homotransplantation of teeth in different stages of development into individuals of different ages, and heterotransplantation (in animals)

Transplantation of stored tooth buds was carried out by (a) autotransplantation of teeth stored no longer than five days (b) homotransplantations following storage of teeth not exceeding five days (c) homotransplantations of teeth stored between five and twenty days and (d) heterotransplantations (animal) of teeth stored between five and twenty days.

Homo- and heterotransplantation of stored tooth buds obtained from dead subjects were performed in animals

At this point the terms tooth bud, preformed tooth and unerupted tooth should be defined, since they will be used in the description of the following experiments. Teeth start to develop from the oral epithelium of the embryo on the fortieth day of gestation and the stages of this development, which are well known, continue well beyond the age of puberty For practical purposes this development may be divided into three distinct though arbitrary stages, based on the following characteristics. 1) The bud is the stage in which the tooth is completely enclosed in the tooth sac. The bud except for the enamel




FIG 133 Development and eruption of a tooth (sketch after Orban) A At the age of three B Five years C Eleven years D Fourteen years

organ, is a soft mass of cells. The enamel organ can be distinguished macroscopically as a harder mass of cells located in the crown of the tooth The cells of the inside layer of this enamel organ are called ameloblasts and play an important role in the further development of the tooth 2) The stage of the preformed tooth. It has its crown fully developed but its roots are just starting to develop 3) The stage of the unerupted tooth The root is almost fully developed but the tooth is still covered (figure 133)

These experiments consisted of eighty-six homotransplantations of tooth buds or preformed teeth. In choosing the donors and recipients according to age the foremost consideration was that they should be of aid in forming solutions to the problems enumerated above Teeth were obtained from donors, six to sixteen years old from whom supernumerary or impacted teeth had to be removed because of orthodontic conditions. Unerupted impacted and supernumerary teeth often obstruct the normal development of the dental arch and therefore confront the orthodontist with formidable problems. Extraction of such teeth is done regardless of the possibilities for transplantation.

In the United States Pafford (30) of Arizona proposed the establishment of a tooth bank in 1953 In Budapest, Hungary the author organized the first tooth bank in 1934 Substantial material was supplied by the Central Institute for Orthodontia, which had 14 000 children under treatment and observation similar institutions throughout Hungary also provided material.

The greatest difficulty encountered in dental transplantation to man has been the lack of sufficient tooth buds and the poor condition in most cases of those that have been available.



FIG 134 Two supernumerary tooth buds at the site of the incisors *Left In situ Right* Four weeks after transplantation at the site of a first molar

It has been necessary to utilize almost exclusively tooth buds that were supernumerary irregularly placed, often misshapen—in short, by no means normal moreover the removal of these tooth buds is a very difficult procedure During the operation it is more important to avoid injury to the surrounding sound teeth than to avoid injury to the superfluous tooth Such an injured tooth should not be used. These unavoidable injuries to the tooth bud may easily account for the occasional unsatisfactory root development following transplantation (figure 134)

Criteria for judging the success of tooth transplantation are 1) Occurrence of infection must be avoided and allergic manifestations or undesirable immunologic reactions which may be harmful even in the event of an otherwise successful transplantation must not be incited 2) The transplant should take perfectly (*a*) It should show complete vascularization (*b*) The periodontal environment should be healthy (no absorption) (*c*) The transplant must form a structurally sound unit with its environment, the mandible and maxilla 3) The tooth must be capable of normal function (4) The periodontal region must show a normal roentgenologic picture 5) The transplant must be esthetically acceptable

Even though very few of the eighty-six transplantations fulfilled the above ideal requirements, a substantial proportion did seem to be successful. This fact encourages continued investigation along the same line and holds out hope that with further organization and refinements in technique we will be able to develop a practical method of tooth transplantation for the clinician.

For easy review the eighty-six cases are listed in tables 4, 5, 6 and 7

The author is continuing his research in order to ascertain the reasons for failures Presumably a tooth bud in the early stages of development is at a disadvantage in comparison to the preformed tooth not only because of the trauma sustained by removal from its environment but also because the adult may be lacking in growth hormones

The results of transplantation of preformed teeth in human are better than those in animals The reason for this is the cooperation with the surgeon in taking care of the transplant lacking in animal experiments. The author has had better results with transplants of preformed teeth than with tooth buds. The reason for this seems to be that the preformed tooth can be put to work much earlier after transplantation than the bud as the patient can be instructed

TABLE 4

| | Age Groups (Years) | | | | | |
|---|---|---|---|---|---|---|
| | 10-15 | 15-20 | 20-30 | 30-40 | 40-50 | 50-60 |
| Number of Cases | 18 | 9 | 14 | 21 | 11 | 3 |

TABLE 5

*Type of tooth transplanted*

| | Teeth | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Buds | [illegible] | Injured | Intact | Fresh | Stored | In maxilla | In mandible |
| Number | 3 | 83 | 45 | 41 | 10 | 6" | 49 | 3" |

TABLE 6

*Length of storage*

| | Days | | | | | |
|---|---|---|---|---|---|---|
| | 1-5 | 5-10 | 10-15 | 15-20 | 20-25 | 25-30 |
| Number | 36 | 22 | 1 | 0 | * | |

TABLE 7

| | Results | | | | |
|---|---|---|---|---|---|
| | Number | Tooth buds | Preformed teeth | [illegible] | [illegible] |
| Successful | 2" | | 2" | 1 | 10 |
| Unsuccessful | 6 | 3 | 3 | * | 4 |
| Doubtful | 30 | | 30 | 1 | 18 |
| Not controlled | 33 | | 33 | 19 | 14 |

to start chewing cautiously very soon after operation.

Since only a small number of usable teeth were available they were used mainly where they did the most good functionally. Thus cosmetic results were only of secondary consideration. Still when the proper tooth was available for the proper site the cosmetic effects were surprisingly good.

## EXPERIMENTAL OBSERVATIONS

Autotransplantation and homotransplantation of teeth yield identical results. In the case of homotransplantation, no difficulty was encountered



FIG 135 Transplanted tooth at the site of a right upper second bicuspid in a 30-year-old person *Left* Before transplantation *Left inset* X ray before transplantation *Right* Eight weeks after transplantation *Right inset* X ray eight weeks after transplantation



FIG 136 Tooth transplant at the site of an extracted right lower canine tooth in a 51 year-old person. *Above* Before transplantation and x ray *Below* Eight weeks after transplantation and x ray

in overcoming the biochemical immunologic effects of tissue incompatibility. These findings would suggest that homotransplantation should be a definite first choice when implanting teeth in humans. The fact that teeth are almost the only tissue that can at present be homotransplanted successfully may be explained, at least in part, by the observation that the tissues of tooth buds have a low protein specificity (Adler). This may explain the slight local or general reaction to the implant (a low protein reaction). In addition, the tissue metabolism of tooth buds is assumed to be much lower than that of any other tissue.

The problem of serologic and immunologic reactions causing tissue incompatibility becomes more significant in cases of heterotransplantation because tooth buds, in spite of low protein specificity, carry a high specificity for the species. This may explain the difficulties encountered in heterotransplantation. Thus far we have no certain methods to overcome the difficulties caused by biochemical reactions in animals. However, very definite and hopeful signs have been observed that these difficulties are not insurmountable.

**Storage**

The results of homotransplantation or heterotransplantation are favorably affected by storage due to the low metabolism of these tissues. The findings in these experiments parallel those in the literature. Carrel (40), in his classic experimental work, expressed the opinion that a successful transplantation presupposes that tissues are transplanted with their full viability kept intact. Many concur in this opinion even today, believing that homotransplants of freshly removed tissue promise much better results than tissue from a "bank." Filatow and Smirin, however, had better results with homotransplant of banked tissues than with those of fresh tissues. We may assume that the process of preservation changes the tissue's biochemical activities by decreasing the antigenic potential and thus neutralizes them, so to speak, minimizing the specific biologic difference between the transplant and the host.

Tooth buds can be successfully stored at +2°C in a solution of Tyrode, merthiolate or Hank's balanced salt solution. In order to store tooth buds longer than twenty days, further experiments regarding their lyophilization are required (39).

The transplanted tooth buds showed continued development but failed to reach maturity. This was particularly noticeable when they were transplanted into older individuals. When the recipients of transplanted tooth buds were younger individuals, the transplants showed signs of more pronounced development and more decided differentiation. Such findings indicate that tooth buds which are transplanted from a young, growing organism carry with them a certain potential for growth and that this potential is soon exhausted unless the recipient organism is capable of replacing it; growth and development come to a standstill. When the operative technique of Fleming was used no growth in the transplant was found without the inclusion of tumor tissue. Therefore, it may be assumed that in Fleming's experiment the growth of the transplant was largely due to a "growth energy" or stimulation that derived from the tumor tissue. (To clarify this question the author subjected the tooth buds to the influence of pituitary growth hormones; it is too early to draw any conclusion from these experiments at this time.) In human experiments the best results were obtained by using unerupted teeth rather than tooth buds. It appears that in such instances the unerupted tooth has enough "growth energy" to develop into a functionally adequate tooth.

The author's experiments showed the same results whether the teeth for transplantation were removed from living or dead animals. In most animal experiments the tooth buds which were successfully used as homotransplants were obtained from freshly killed animals. Thus a source for homotransplantation material in hu



FIG. 137. Human tooth bud transplanted at the site of the first molars of the mandible in an 8 month old dog three weeks after transplantation.

mans may eventually be found by utilizing teeth obtained from suitable cadavers of children. Tooth banks may be established following the pattern of blood banks and banks for other tissues.

In heterotransplantation, unwanted biochemical reaction and tissue incompatibility play a significant role. Although there are indications pointing to the possibility of overcoming such difficulties, no conclusion may be reached on the basis of experiments thus far performed

## CONCLUSIONS

1) The most suitable teeth for homotransplantation are the ones in the preformed and in the unerupted stage of development, where there is a fair amount of root formation which is still in progress.

2) Tooth buds, where the tooth is just beginning to differentiate, are much less suitable for homotransplantation.

3) Results in host tissues other than the mandible and maxilla are very poor

4) Teeth that were injured in the transplanting process may take but their further development will be arrested

5) Further development of transplanted teeth is more pronounced in young subjects than in adults.

6) The blood group the Rh factor tissue incompatibility or unwanted immunobiologic reactions play an insignificant role in homotransplantation of teeth.

7) Successful results are enhanced by careful surgical technique and early careful handling of the transplant.

Since the first tooth transplanted in these experiments has been *in situ* only a relatively short time it is not yet possible to express a definite opinion on the ultimate fate of tooth transplants and consequently on the value of



FIG 139 Transplanted bicuspid at the site of an extracted first molar of a 13 year-old boy



FIG 140 *Left* Incisor tooth transplanted at the site of a right lower canine tooth in a 58-year-old person *Right* One and a half years after transplantation



FIG 138. *Above* Four months after transplantation of a tooth bud into the tibia of a dog *Below* Transplanted tooth bud into the ear lobe of a guinea pig

the method employed Successes up to the present time can thus be considered only as partial successes Experiences in this field have constantly led to new problems for the solution of which further experiments are necessary

The number of teeth successfully transplanted by the author is not large *per se* but compared to figures reported in the literature it is considerable *perhaps even unique On the basis of* these experiments as well as clinical experience the conclusion seems clear that the transplantation of tooth buds is a suitable method to replace lost teeth.

The author wishes to express his deep appreciation to the Rockefeller Foundation which made the continuation of these studies in the United States financially possible also to Dr Lyndon A Peer and his laboratory staff at St Barnabas Hospital in Newark whose cooperation was of great help

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# PART VII

# *Blood Vessels*

CHAPTER 11*

# *Transplantation of Blood Vessels*

RALPH A. DETERLING, JR.

## AUTOGENOUS BLOOD VESSELS

### Transplantation of Fresh Artery

#### *Experimental Studies*

Probably the earliest studies of free arterial transplantation were those reported in 1896 by Jaboulay and Briau (1). In the course of studying the suture technique of Jassinowsky, these authors, employing interrupted mattress sutures in the resected carotid arteries of dogs, divided the artery in two sites on occasion. The isolated segment was recognized as constituting a true graft.† Unfortunately, clots formed in all experiments on the third or fourth day. When examined a day or so later, the grafts appeared viable and it was noted that the clot formed at the site where clamps had been applied to halt blood flow.

In 1903 Höpfner (2) described the first functional success with fresh carotid reimplantation in dogs. He employed a non-suture technique with a metallic prosthesis similar to that designed previously by Payr. In the same year Exner (3), using the magnesium rings of Payr, transplanted segments of carotid and femoral arteries to the contralateral artery, but all failed because of thrombosis. In 1908 Capello (4) reported success with fresh autogenous arterial grafts in association with experiments on organ transplantation. In the experiments by Borst and Enderlen (1909) (5) there were three successful grafts of carotid artery—one a reimplantation and two transplants to the contralateral artery. When these specimens were examined at 10, 14 and 122 days there was no dilation and only minimal thickening was noted near the suture lines. Microscopically, this thickening was composed of connective tissue and newly formed elastic fibers. The muscular elements appeared to be unchanged. In the same year, Stich and Zoeppritz (6) observed the same intimal thickening in a fresh arterial transplant of femoral artery into the carotid artery of the dog examined at 14 days. In 1910, Guthrie (7) and Villard (8) reported independently on experimental transplantation of blood vessels. The former found a successful contralateral carotid arterial graft entirely normal in appearance at 28 days. Villard found no significant alterations in a functioning carotid arterial reimplantation at 93 days.

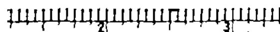
Yamanouchi (9) in 1911 published a comprehensive and very detailed experimental study of various types of vascular transplants, making observations on four successful fresh arterial grafts functioning for 7 to 108 days. The earliest specimen was an abdominal aortic segment, and the oldest, a carotid reimplantation. The other two were carotid arteries excised at 86 and 95 days and transplanted to the femoral artery. In addition to observing fibrous thickening of the intima, Yamanouchi described new elastic fibrils arising from the site of anastomosis. Von Faykiss (1912) (10) described his results with various types of grafts and had moderate success with fresh autogenous arterial transplants. In 1913 Castiglioni (11) claimed 71.5 per cent success with artery to artery trans-

* Supported by grants from the New York and American Heart Associations.

† "*Dans quelques cas, par une double section suivie d'une double suture, nous avons rendu indépendant et isolé un segment artériel réalisant ainsi une véritable greffe.*"

plantations or reimplantations observed for 63 to 250 days. He too observed intimal thickening and new elastic fibers in the region of the suture lines in most specimens. In one studied at 250 days intimal thickening was present throughout the length of the graft. By contrast Carstaglioni had only 25 per cent success with arterial segments transplanted into veins and observed for 105 days. Also in 1913, Moure (12) reported a *normal femoral arterial graft* transplanted into the carotid artery for 91 days.

*For many years then, few if any experiments* were conducted, because of the clinical applicability *of autogenous veins for peripheral arterial* lesions. However, when general interest in vessel *transplantation was reawakened by the clinical* success with preserved arterial homografts, a *few studies were reported.* Miller and his colleagues (1951) (13) studied the function of small arterial transplants and had fine results with five autogenous femoral arterial transplants. In 1952 Deterling and his associates (14) reported 100 per cent functional success and minimal, if any, structural changes in eight carotid arteries inserted into contralateral arteries. Other types of grafts were evaluated as well and the effectiveness of different suture materials was studied. Continuous sutures of 00000 chromic catgut and 00000 braided silk were employed. In unreported experiments, Deterling and Olsen (1952) (15) transplanted fresh autogenous carotid segments to contralateral vessels simultaneously



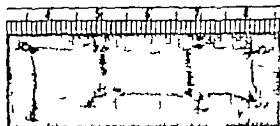


Fig. 141A. Fresh autogenous canine carotid *arterial graft in contralateral artery. (Gross ap*pearance of graft at one month. There is complete endothelization of anastomoses and transplant with no mural thrombus. There has been no change in length or diameter but the thickness of the graft is slightly less than that of an adjacent artery. See photomicrograph.

with insertion of rat and rabbit aortic heterografts. As in the other series, the autogenous arteries could scarcely be distinguished *from the* adjacent artery (fig. 141). In the same year Parson, Gerbode and Cox (16) reimplanted eight segments of abdominal aorta in dogs in a study aimed at clarification of factors contributing to degeneration of vascular graft. Silver foil was wrapped about the external surface of five of these segments to impede revascularization of the graft. One of these thrombosed at nine month. In all, including controls, there were intimal thickening and persistence of cellular *elements.* No destruction of the elastic fibers nor calcification was observed in 18 days to 9 *months. The authors concluded that the delayed* revascularization did not produce significant *degenerative changes. In 1953 Henrotin and* Veroft (17) made an extensive evaluation of *vascular grafts and claimed good results with* six arterial autografts in dogs.

Brown *et al.* (1958) (17A) reported an 82 per cent patency of long carotid autografts in dogs studied at one year in contrast to only 30 per cent success with *freeze-dried* carotid autograft.

In studying the response of vascular grafts to growth stimuli, Johnson and his associates (1951) (18) reimplanted aortic segments in growing pigs. There was no significant difference in the diameter of the graft and the host aorta. Griffith and his colleagues (1955) (19) also confirmed the growth of autogenous aortic graft in growing pigs but observed calcification in one specimen examined at about eight months. In 1952 and 1953 *Sauvage and Harkins (20-23) summed up* the extensive investigations of various types *of grafts by the latter and his associates. In accord* with other observations Sauvage and Harkins *found persisting integrity of cellular elements and* elastic fibers. In the growing animal there is the persistence of viable elements observed in mature animals but occasionally degenerative changes may develop.

The reports compiled *from* the literature indicate that the gross appearance of autogenous arterial transplants very closely resembles that of the recipient artery. Some thickening of the intima, usually adjacent to the anastomoses, may occur. Although earlier investigators suggested the formation *of fine elastic fibers in these zones* most of the recent studies have failed to confirm *the development of new elastic tissue and ascribe* the intimal thickening primarily to connective

tissue growth. Although some thickening of the adventitial zone by fibroblasts occurs, it is commensurate with normal healing processes. Of importance is the fact that the autogenous arterial transplant does not become thinned, dilated or aneurysmal with time. In 1955 Khrustitch (24) described an experimental study of the degeneration and regrowth of nerve fibers into arteries and arterial autografts after section and anastomoses. By the eighth day he observed a growth of new nerve fibers in the adventitia, and by the fifteenth day he noted a variety of configurations had developed at the end of nerve fibers in contact with the vessel wall. By the

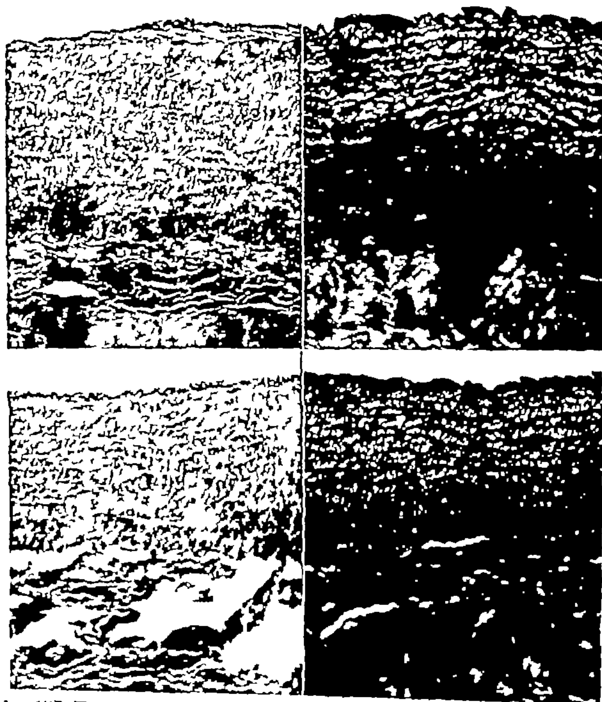


FIG. 141B. The upper photomicrographs show normal canine carotid artery. Those of the autogenous arterial graft (lower set) reveal minimal fibrosis of the adventitial zone and persistence of cellular elements of the vessel wall. The elastic fibers are unchanged from those in the normal artery although the thickness of the wall is slightly less. (Hematoxylin and eosin; Verhoeff stain.) × 11?

fiftieth day the graft contained new nerve fibers often naked axon cylinders penetrating the wall and scar tissue.

Further understanding of the histologic pattern of the healing of autogenous vessels has been contributed by Jacobellis and Taddeini (1955) (25). Mortensen and his coworkers (1955) (26) and many others Hammer, Seay and Hill (1953) (27) reinserted segments of aorta of dogs, and reinforced the wall with pedicle grafts of ileum from which the mucosa had been stripped. The technique was developed to reduce chances of aneurysm formation in grafts.

In 1957 Macpherson and Duthie (27A) reported on the excellent functional and histological status of fresh autogenous aortic grafts in 11 dogs studied up to thirteen months. There was an uptake of $S^{35}$ in the graft similar to that in the host aorta.

In view of the ideal characteristics of the autogenous arterial graft, several investigators have described techniques whereby short segments with a diameter approaching that of the aorta could be fashioned from expendable smaller arteries such as the internal mammary or even the subclavian artery. In 1951 Hurwitt and Sandblom independently developed a technique whereby a length of artery was divided and each segment then incised longitudinally to produce a "panel." The edges of the panel could then be approximated to produce a graft of adequate caliber. By this method the diameter could be increased with additional panels. In a three-year study Hurwitt and Kantrowitz (1953, 195 ) (28, 29) found preservation of the media and satisfactory function in aortic transplants prepared from the splenic artery of dogs.

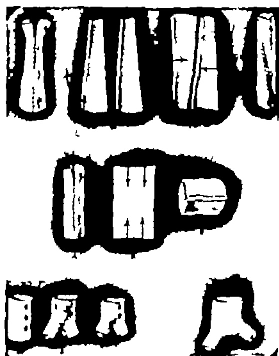


Fig. 112. Various techniques for producing autogenous arterial grafts of large caliber from small expendable arteries are shown. Additional methods for creating bifurcation grafts from straight segments are also demonstrated. (Courtesy of A. A. Lazzarini, Angiology 4: 516, 1953.)

In 1952 Sandblom and his coworkers (30) described a similar technique by which a large caliber graft could be produced. A long segment of expendable artery was incised longitudinally and the opened ends of the vessel were approximated. Thus a large caliber could be produced with one suture line. However, the length of the new graft was limited by the circumference of the original arterial segment. This same technique was described independently by Lazzarini (1953) (31) and by Potts, Albert and Fischer (1953) (32) (fig. 112). A subsequent report in 1956 by Muren (33), one of Sandblom's associates, indicated that the viable elements persisted and that good function had continued in dogs during a two-year period of observation. Potts described the actual measurements of grafts of this type which could have been produced in two patients with coarctation but they did not require a graft for surgical correction.

Schmitz and associates (1953) (34, 35) described the use of panel type grafts of subclavian artery transplanted into the thoracic aorta of adult dogs and growing pigs. These investigators observed patency persisting during a study period of six months, and satisfactory growth of the grafts in the pigs. In 1955 Griffith and his group (19) reported a technique for producing autogenous arterial grafts of wide diameter by means of alternating long strips of autogenous artery with strips of Vinyon-N taffeta fabric (fig. 113). They observed growth of the autogenous strips in growing pigs and noted satisfactory function. It was in one such graft that calcification was observed, as mentioned earlier in the autogenous arterial tissue.

In a study of factors influencing the degeneration of grafts, Creech and associates (19 ) (36) fed a high cholesterol diet to dogs some of which had been rendered hypothyroid by radio-

active iodine. In animals with a serum cholesterol of more than 1000 milligrams per cent the host aorta and autogenous aortic grafts were equally involved by atherosclerotic lesions. In animals with levels below 1000 milligrams per cent, the grafts were unaffected.

Infrequent experiments have been reported concerning the insertion of autogenous arterial grafts into veins. In general the functional results have been disappointing. In 1909 Borst and Enderlen (5) transplanted a carotid segment into the jugular vein but thrombosis occurred

*Clinical Use*

Because of the unavailability of suitable material or because of reluctance on the part of surgeons to sacrifice a major artery to produce a graft of sufficient caliber, there have been only rare reports of clinical application of autogenous arterial transplants. Bricker (1954) used fresh autogenous grafts of superficial femoral artery to bridge 7 to 8-inch defects in the iliac arteries of three young patients and got good results. On rare occasions a surgeon has used the subclavian artery as a free autogenous transplant to bridge the gap between the aorta or subclavian artery and the pulmonary artery in the treatment of tetralogy of Fallot. Even less frequently has such a graft been employed in the repair of coarctation of the aorta.

### Transplantation of Fresh Vein

*Experimental Studies*

In 1898 Gluck (37) performed the earliest experiments with free autogenous venous grafts but all failed because of thrombosis. In 1903 Exner (3) and Höpfner (2) independently described experiments with autogenous venous grafts in dogs but these also failed because of thrombosis. In 1905 Carrel and Guthrie (38) described their joint studies of this type of graft. They achieved the first success with free autogenous venous grafts, employing the suture technique described by Carrel in 1902. Additional experimental success with the 'biterminal, complete venous graft was reported subsequently during the years 1906 to 1912 by both authors (39–47) Their initial success had been achieved through the insertion of the external jugular vein into the carotid artery or the femoral vein into the femoral artery

In 1906 Carrel emphasized the adaptability of the wall of venous transplants to arterial pres-

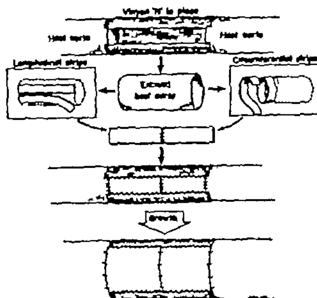


FIG 143 Method for producing compilation grafts with alternating strips of synthetic fabric and autogenous artery The strips of artery may be of varying length depending on the number of panels employed and these are derived from longitudinal or circumferential sections of the arterial segment (Courtesy of C A Griffith and associates and by permission of Surg Gynec & Obst 101:225 1955)

sures and described experiments purporting to demonstrate 'arterialization" of veins functioning in the arterial system. In a 14-day specimen of jugular vein in the carotid artery, significant thickening of all layers by connective tissue was observed. In 1910 Carrel described two successful transplants of autogenous inferior vena cava to the abdominal aorta of cats. In one studied at 32 days there was thickening of all layers by connective tissue and the media appeared to be predominately connective tissue. In the other graft, removed at 303 days, an occasional elastic fiber was observed, but marked thickening of all layers by connective tissue was the major alteration. A transplant of inferior vena cava to the abdominal aorta of a dog was studied at 415 days, and an increase in smooth muscle cells and fibrous tissue of the media was described. The adventitia was greatly thickened by connective tissue, but the intima had thickened only slightly

Also in 1910 Carrel described progressive alterations observed in a jugular vein transplant in the carotid artery At 143 days he noted a thickening of the media as a result of an increase in fibrous tissue and muscle cells. At 605 days the graft exhibited still more sclerosis and no muscle

cells persisted. Carrel attributed the earlier changes to functional hypertrophy and pointed out the sclerotic nature of the alterations resulting from continued arterial pressure. In the same year Guthrie described marked adventitial fibrosis of a jugular graft in the carotid artery removed at 28 days. He found no muscular elements remaining.

In 1906 Goyanes (48) implanted segments of inferior vena cava in the abdominal aorta of fifteen dogs. In the one successful graft among them, examined at 13 days, there were no significant alterations except for a fibrinous deposit over the suture lines. Watts (1907) (49) presented an extensive review of the literature relating to suture technique and transplantation of blood vessels and organs. In this report he described personal experiments including one successful transplant of jugular vein to carotid artery in a dog, studied at 26 days. Watt observed some thickening of all layers of the graft as a result of connective tissue growth.

Stich, Makkas and Dowman in 1908 (50) described rather extensive experiments with blood vessels from the surgical clinic of Professor Garrè of Bonn. They reported twelve experiments in which veins were transplanted into arteries with five remaining patent. Their longest observation was 211 days. The next year Stich and Zoeppritz (51) described the histologic changes observed in three grafts of jugular vein into carotid artery studied at 26, 211, and 409 days. In all there was significant fibrous thickening of the intima with new elastic fibrils. The intimal thickening appeared to be irregularly disposed in the 200-day specimen and was much greater near the suture lines than in the mid-portion of the 409-day graft. The media and adventitia seemed relatively unchanged except for stretching of the elastic fibers.

In 1909 Borst and Enderlen (5) reported thrombosis in three of five jugular vein grafts in carotid arteries observed up to 82 days. In a 10-day graft they described thickening of the intima, mainly near the suture line. They observed new elastic fibers as well as smooth muscle cells in the thickened area. Also in 1909 Fischer and Schmieden (52) described their findings in seven jugular vein grafts in carotid arteries observed from 10 to 86 days. They observed occasional subintimal thickening near the suture lines but did not find new elastic tissue. There was an increase in connective tissue in the adventitia.

Villard (1910) (8) described a jugular vein graft functioning in the carotid artery for 113 days. He found subintimal thickening with new muscle cells. No alterations were present in the media but an increase in connective tissue thickened the adventitia. Yamanouchi (1911) (9) described the histologic alterations in a graft of femoral vein to femoral artery functioning for 85 days as well as the changes observed in three jugular vein grafts to carotid artery functioning for 51, 66, and 207 days. Yamanouchi observed a marked increase in connective tissue of all layers and described in addition new elastic fibers in the intima and an increase in muscle cells of the media. He believed the intimal changes occurred as a result of increased arterial pressure.

In 1911 Curcio (53) also described fine elastic fibers and fibrous thickening of the intima adjacent to the anastomoses of a jugular vein graft functioning in the carotid artery for 23 days. He also observed that the intima was less thickened in the mid-portion of the graft. In the same year Palazzo (54) described the morphology of five jugular vein grafts in the carotid artery functioning for 10, 50, 100, 475, and 483 days. Although no significant alterations were observed at 10 days, there appeared to be an increasing new subintimal zone of connective tissue as well as hypertrophy and hyperplasia of the muscle cells of the media. Palazzo also described some increase in the collagen and elastic fibers of the media.

In 1912 von Fackse (10) commented on his poor functional results with experimental venous grafts in arteries. In 1913 Castiglioni (11) added his observations regarding twelve jugular or femoral vein grafts in the carotid artery. Three successful ones were studied at 70, 77, and 105 days. This author described a thickened intima with new elastic fibers and an increase in endothelial cells. The media demonstrated hyperplasia and hypertrophy of the smooth muscle cells and the adventitia was thickened by connective tissue. In the same year Moure (12) described an interesting experiment involving a segment of jugular vein in the carotid artery. After 801 days a 1-centimeter segment was excised from the mid-portion of the functioning graft and a comparable segment from the

contralateral jugular vein was implanted into the defect. The excised segment revealed a new subendothelial zone and marked thickening of the entire wall. After 37 days, the graft was re-explored and the new segment appeared as thick as the original graft. Moure concluded that this was a demonstration of persisting viability and adaptation. In 1917 Goodman (55) implanted several vessel grafts while studying suture technique. In a transplant of femoral vein to carotid artery studied at 8 days, the author described degenerative changes of the wall.

Clinical application of these many findings which demonstrated the functional success of autogenous veins in the arterial system found widest acceptance among the German military surgeons for the treatment of aneurysms during and following World War I—Bier (56, 57), Lexer (58, 59), Subbotitch (60), von Bonin (61), von Haberer (62, 63) and Weglowski (64). It is somewhat surprising that no further experimental studies of note were to be reported for more than two decades. Indeed, it was out of the anticipated needs for a simplified method of vascular anastomosis that Blakemore and his associates in 1942 (65) and 1943 (66) described a non-suture technique employing vitallium tubes similar to those described in 1903 by Höpfner. In the experiments of these investigators autogenous femoral vein was transplanted into the corresponding artery in dogs with moderate functional success. In 1945 additional studies (67, 68) involving use of inferior vena cava in the abdominal aorta produced better results.

Donovan (1949) (69) by-passed the pulmonary valves by autogenous venous grafts between left pulmonary artery and right ventricle but without lasting success. Following the introduction of preserved aortic homografts into clinical usage by Gross and his colleagues, increasing numbers of experimental studies evaluating the behavior of autogenous veins in the aorta appeared.

In 1949 Johnson (70) reported on the behavior of segments of inferior vena cava in the abdominal aorta in dogs observed for periods up to 14 months. This study demonstrated nicely the fibrous thickening which became progressively greater with the passage of time but significant dilation—first mentioned by Höpfner (2) in 1903—was also described. In subsequent studies (18) reported in 1951 the transplantation of autogenous vena cava into the aorta of growing pigs demonstrated that the growth of such grafts could equal that achieved by the host aorta. Still later (71) in 1953, transplants of vena cava were inserted into the thoracic aorta of growing pigs with and without an external support of autogenous pericardium. Because of the marked dilation and even aneurysm of grafts, Johnson and his coworkers cautioned against the use of venous grafts in the thoracic aorta of patients.

A series of fourteen experiments was begun by Coleman, Parshley, and Deterling (72) in 1951 which completely confirmed the studies of Johnson and his associates on the behavior of fresh autogenous inferior vena cava in the aorta of dogs. A progressive thickening of the autogenous vein graft was observed as a result of an investment of the adventitial layer with fibroblasts of the host. This process produced some thickening of the intimal zone adjacent to the aorta. As was demonstrated by Johnson, the wall of the graft was often four times the thickness of the original vein by 14 months.

In prolonged observations, however, Coleman and Deterling noted thinning of the graft after 15 months, although there was individual variation in the rate of alteration. In all dogs studied longer than this period there was continued patency but dilation developed. Aneurysm of the graft was observed in two animals at 39 months. The histologic pattern consisted of an early fibroblastic response by the host which was most intense during the first six months. At no time was there conclusive evidence either of hypertrophy or of new formation of smooth muscle cells or elastic fibers. The subsequent thinning resulted from cicatrization of the new fibroblastic layer as well as attenuation of the original graft. In time gradual disappearance of muscle cells and elastic fibers was observed (figs. 144–146).

In 1951 Sako (73) reported on the use of autogenous caval grafts in the aorta and observed that excessive dilation appeared to be controlled by external support with autogenous fascia or skin. Some theoretical objections were raised with respect to the latter material for clinical cases. In 1953 Sako and Varco (74) extended the investigation of autogenous venous grafts and concluded that when inserted into the thoracic aorta, the grafts invariably dilated with time



FIG 144 Fresh autogenous inferior vena caval grafts in the aorta of dogs *Above* Specimen in abdominal aorta for one month There has been fibrous thickening of the graft and little or no dilatation The surface is well endothelized *Below* Aortogram of graft in thoracic aorta for 2½ years The cava has elongated and developed fusiform dilation

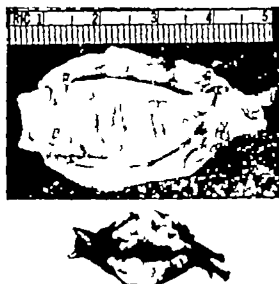



FIG 145 *Above* Specimen in abdominal aorta for 30 months The graft has become greatly dilated and very thin *Below* Transillumination of specimen shown above emphasizes the thinning

Anzola and associates (75) in 1952 published a comparative study of long small caliber grafts of various types in dogs In 20 experiments with autogenous venous grafts 75 per cent were patent at 16 weeks The authors concluded that autogenous veins were the most successful of all the types studied Everson (1951) (76) implanted four caval grafts into the abdominal aorta of growing pigs and observed growth and some dilation in the two which remained patent. Weis (1951) (77) found early dilation of vein grafts in the thoracic aorta of dogs

Nabatoff and his associates (78 80) conducted a long term study of maximal length vena caval transplants in the abdominal aorta of large dogs In their initial report (1953) they described progressive dilation occurring after 12 months and becoming aneurysmal at 2 years in most of the animals. A report in 1955 evaluated their four year studies of these grafts (80) The authors described the transplants as thin-walled sacs lined with endothelium. Although considerable aneurysmal dilation had developed by the second year there was minimal dilation subsequently and none of the grafts ruptured.

In France Oudot (1951) (81) implanted caval grafts into the thoracic aorta in 27 dogs with 14 survivors. He observed thickening of the vein within 3 or 4 weeks and reported one small aneurysm present at three months In Italy de Sanctis (1952) (82) described the histologic appearance of anastomoses of jugular vein grafts in the carotid artery Tertitta in 1954 (83) described experiments in which 12 femoral vein grafts were transplanted into the artery Excellent results were obtained with 9 of them. In Belgium, Henrotin and Veroft (1953) (1 ) described extensive experiments in dogs among which were studies of 32 venous grafts and 11 bifurcations of vena cava into corresponding arteries. Although some satisfactory results were recorded concerning the grafts only one of the bifurcations succeeded Kristinov and Ikeler (1955) (84) also reported studies of vascular replacement by arteries or autogenous vein

As part of very comprehensive studies of vascular transplants by Harkins and his associates Schmutz and his associates (1953) (31 3 85) described their studies with fresh caval grafts inserted into the abdominal aorta of 36 adult dogs and 12 growing pigs Significant early thrombosis was seen in the dogs and aneurysmal

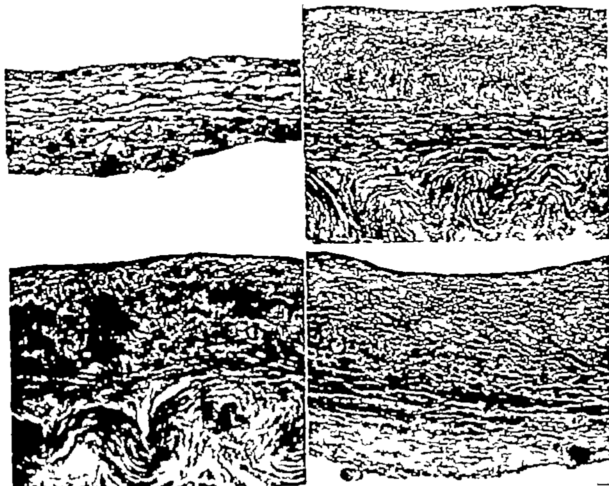


FIG 146 Photomicrographs demonstrate the differences in caval grafts functioning 6 hours (*upper left*) 6 weeks (*upper right*) 3 months (*lower left*) and 30 months (*lower right*) There is marked fibroblastic thickening following the first week of implantation as shown at 6 weeks and 3 months Subsequently there is a slow condensation of the new intima and adventitia with areas of hyaline degeneration Ultimately the elastic fibers appear to be reduced in number (Hematoxylin and eosin Verhoeff stains × 45

dilation occurred in many pigs after 6 months. In others, growth of the graft did not keep pace with the host aorta The authors believed that graft complications were likely to occur if there was biterminal disproportion in diameter exceeding 21 per cent and if the graft was longer than 5 centimeters. Nyhus and associates (1955) (86 87) added additional observations regarding the behavior of autogenous cava in the thoracic aorta of growing pigs. The dilation and formation of aneurysms were not related to the length of the graft. Thrombus formation was very uncommon and calcification was never observed. At 8 months viable components of the original graft could be recognized histologically but some degree of medial degeneration was also evident.

In 1958 Jones and Dale (87A) described results of shunt grafts of autogenous vein in the femoral artery of dogs and noted persistence of a normal pulse pattern. There was no loss of smooth muscle but there was a disappearance of elastic tissue and development of fibrosis of the graft wall Sixty per cent of the 42 grafts remained patent

Studies of the endothelial structure of a jugular vein graft in the aorta of a dog were reported by Bollack and his coworkers (1954) (88) By silver nitrate staining they demonstrated that the endothelial pattern in the graft differed from that of the aorta German and Black (1954) (89) demonstrated the weakness of venous tissue when they produced aneurysms by inserting a blind segment of jugular vein into the side of the carotid artery

Some investigators have studied various materials for external support of venous grafts



FIG. 144. Fresh autogenous inferior vena caval grafts in the aorta of dogs. *Above*: Specimen in abdominal aorta for one month. There has been fibrous thickening of the graft and little or no dilatation. The surface is well endothelized. *Below*: Aortogram of graft in thoracic aorta for 2½ years. The cava has elongated and developed fusiform dilation.

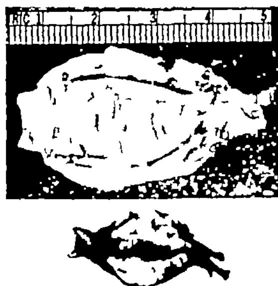


FIG. 145. *Above*: Specimen in abdominal aorta for 39 months. The graft has become greatly dilated and very thin. *Below*: Transillumination of specimen shown above emphasizes the thinning.

Anzola and associates (75) in 1952 published a comparative study of long, small caliber grafts of various types in dogs. In 20 experiments with autogenous venous grafts 75 per cent were patent at 10 weeks. The authors concluded that autogenous veins were the most successful of all the types studied. Everson (1951) (76) implanted four caval grafts into the abdominal aorta of growing pigs and observed growth and some dilation in the two which remained patent. Weiss (1951) (77) found early dilation of vein grafts in the thoracic aorta of dogs.

Nabatoff and his associates (78-80) conducted a long term study of maximal length vena caval transplants in the abdominal aorta of large dogs. In their initial report (1953) they described progressive dilation occurring after 12 months and becoming aneurysmal at 2 years in most of the animals. A report in 1955 evaluated their four year studies of these grafts (80). The authors described the transplants as thin-walled sacs lined with endothelium. Although considerable aneurysmal dilation had developed by the second year, there was minimal dilation subsequently and none of the grafts ruptured.

In France Oudot (1951) (81) implanted caval grafts into the thoracic aorta in 27 dogs with 14 survivors. He observed thickening of the vein within 3 or 4 weeks and reported one small aneurysm present at three months. In Italy de Sanctis (1952) (82) described the histologic appearance of anastomoses of jugular vein grafts in the carotid artery. Ferlitta in 1951 (83) described experiments in which 12 femoral vein grafts were transplanted into the artery. Excellent results were obtained with 9 of them. In Belgium Henrotin and Veroft (1953) (1 ) described extensive experiments in dogs among which were studies of 32 venous grafts and 11 bifurcations of vena cava into corresponding arteries. Although some satisfactory results were recorded concerning the grafts, only one of the bifurcations succeeded. Krestinov and Boikov (1955) (84) also reported studies of vascular replacement by arteries or autogenous veins.

As part of very comprehensive studies of vascular transplants by Harkins and his associates, Schmitz and his associates (10, 3) (31-35, 85) described their studies with fresh caval grafts inserted into the abdominal aorta of 36 adult dogs and 42 growing pigs. Significant early thrombosis was seen in the dogs and aneurysmal

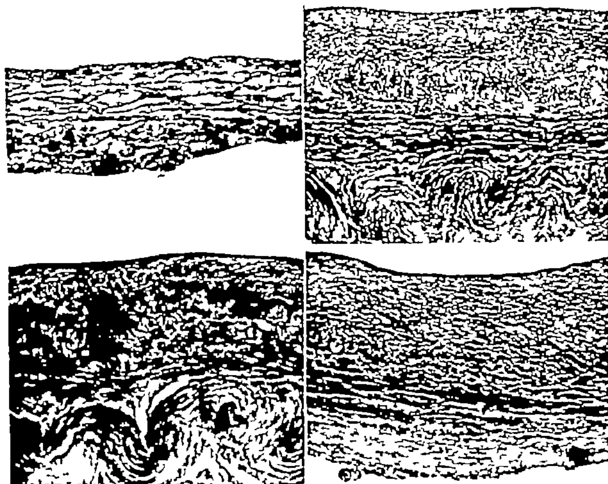


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Some investigators have studied various materials for external support of venous grafts

in an attempt to control dilation. In 1951 Sako was encouraged with his results with autogenous fascia or skin. In 1953 Varga and Deterling (90) described the use of a tulle net of monofilament nylon yarn wrapped about grafts of external jugular vein in the aorta of dogs. Although control (unsupported) grafts dilated significantly within one year, the protected grafts showed no significant change for a period of two years. However, subsequent rupture of the nylon yarn at the knots was observed and pronounced dilation developed rapidly in three animals. The authors considered these failures to be the result of a marked loss in tensile strength in the nylon. Schmitz and his colleagues (1953) (35) described a plication technique for reducing the diameter of venous grafts at time of implantation. Zech and associates (1954) (91, 92) tested the effect of external application of dicetyl phosphate to venous grafts and noted limited linear growth of treated segments in thoracic aorta of growing pigs.

*Chun, Forney and Fell (1954) (93, 94)* implanted jugular vein grafts over lucite or polythene tubes in the fascia of the external oblique muscle. After a few days to weeks the reinforced veins were inserted as aortic replacements. No dilation was observed up to 140 days except in one animal. Egdahl, Hume and Schlang (1955) (95) demonstrated the importance of surrounding tissues coming in contact with vein transplants. Venous grafts encased in solid polythene tubes thrombosed in 70 per cent of the experiments, whereas only 13 per cent occluded if the tubes were perforated. In 1955 Southgate, Fomon and Mahoney (96) reported that dilation of autografts of vena cava implanted into the thoracic aorta of dogs was not controlled by an external wrapping of Ivalon sponge. Significant alterations were noted at 391 days.

In 1956 Zech *et al.* (96A) described the use of a living intercostal muscle bundle about fresh autogenous caval grafts in the thoracic aorta of growing pigs. There were three aneurysmal grafts and in a dilated graft bone formation replaced the muscle. Nyhus *et al.* (96B) reported on the failure of liquid latex or plication to restrict development of aneurysm of venous autografts in growing pigs.

*Clinical Use*

Probably the first use of a venous graft in the treatment of human disease was by Goyanes in 1906 (48). He employed a segment of autogenous popliteal vein *in situ*, attaching the proximal and distal ends to the corresponding ends of the concomitant artery after the excision of an aneurysm. This was not a free graft since the functioning branches of the venous segment were left undisturbed. In the previous year this author (97) reported a series of experiments in dogs in which success had been achieved with vena caval grafts in the aorta. Also in 1905 Carrel (98, 99) advocated the use of venous grafts in the treatment of aneurysms and traumatic lesions of arteries in man. He claimed to have been the first to achieve functional success with free venous grafts experimentally. His experience with them was reported in detail in collaboration with Guthrie (7, 38, 39).

In 1907 Lexer (100) inserted what is generally conceded to be the first free venous graft clinically. He used an 8-cm. segment of saphenous vein for the reconstruction of the left subclavian artery after excision of an aneurysm. The patient died subsequently and the graft was patent. A small mural thrombus had formed at the site of the arterial clamps. Lexer subsequently (1912, 1916, 1921, 1925) (58, 59, 101–103) reported on cases in which venous grafts were used for subclavian aneurysm with good results.

In 1911 Mantelli (104, 105) used a venous graft for the repair of the femoral artery following excision of a sarcoma. In the same year venous grafts were employed also by Palazzo (51) and by Pirovano (106). The latter author referred to an earlier case treated by Doyen. In 1917 Pringle (107) described his use of venous grafts in a patient with a popliteal aneurysm and in one with an aneurysm of the axillary artery. One patient had a patent graft when he died three years later. Coenen (1913) (108) successfully used a venous graft to reconstruct the femoral artery involved in a gunshot wound. In 1914 Touraine collected from the literature thirteen clinical cases from the literature in which grafts had been used with six known excellent results [Fontaine (109)].

During World War I, very few arterial injuries were treated definitively with grafts. The most extensive experience appears to have been in the German army, and several reports describe encouraging results [Subbotitch (60) and von Haberer (62, 63)]. Bier (101) (45, 46) discussed the management of peripheral

aneurysms. Autogenous venous grafts were employed in three cases with persistent function in two. Lexer (1916, 1917, 1925) (58, 59, 103) reported on clinical results with venous grafts and described a successful 5-year follow-up of a 16-cm. graft used in the right iliofemoral artery after excision of an aneurysm. He collected 51 cases from the literature, of which 39 were successful. Lexer mentioned one case in which an associated injury of a major vein was bridged also by a venous graft. In his later report he mentioned 13 personal cases. In 1917 Warthmüller (110) discussed 52 cases collected from the literature. Weglowsky (1925) (64) described 51 cases of aneurysm which were not in Lexer's report.

During the two decades following World War I there were few if any reports concerning the clinical use of venous grafts. In 1937 Karitsky (111) described excellent results with the use of saphenous vein in two patients with aneurysms of the femoral artery resulting from gunshot wounds. One patient was followed for over nine years. Rehn (1942) (112) described the results with saphenous vein in five patients with aneurysms of the subclavian artery. In one, the graft was too small and the author suggested more frequent use of the superficial femoral vein. In 1944 he (113) reported on five patients with gunshot wound of the carotid artery and described satisfactory results in four of them. Killian (1943) (114) commented on seven patients with aneurysms resulting from gunshot wounds. All were successful except two with partial failure resulting from technical problems. Franz (1944) (115) collected 46 cases of peripheral arterial aneurysms in which only 14 of the venous grafts remained patent.

In 1942 and 1943 Blakemore, Lord and Stefko (65, 66) described experimental studies with venous grafts aimed at the development of a non-suture technique which might permit wider use of grafts by military surgeons during war. They later (1945) described the successful application of the technique in three patients (67, 68). The procedure called for everting the ends of a venous graft over a flared short tube of vitallium. With the vein secured by a ligature the tube could be introduced into the cut end of an artery and secured simply by another ligature. In 1945 Blakemore and Lord proposed that prepared segments of vein could be sealed and quickly-frozen in glass tubes with saline and stored in a vessel bank under refrigeration (fig. 147). Although supplies of the vitallium tubes were acquired by the armed forces, they saw little use clinically. The interesting fact that in very few cases of arterial injury occurring during World War II were the patients treated by any kind of grafting procedure was emphasized in the comprehensive report on vascular surgery in the armed forces by DeBakey and Simeone (1946) (116).

In 1947 Bätzner (117, 118) collected 63 cases wherein vein grafts were used in the treatment of traumatic aneurysms of peripheral arteries. Of interest was one patient with excellent function 22 years following operation but with aneurysmal dilation of the graft. The dilated segment was excised with successful anastomosis. The graft had been obtained from a vein in the region of an arteriovenous fistula, and the author felt that this explained the apparent weakness of the graft.

Aneurysms of the popliteal artery were studied by Blakemore, and in 1947 he described a reparative technique whereby a venous graft, after preparation with vitallium tubes, was inserted into the ostia of the afferent and efferent arteries within the opened sac as an inlay vessel. The wall of the sac was then imbricated about the graft for external support (119). In 1949 eight successful venous grafts in occlusive disease were reported by Leriche and Kunlin (120), and in the same year Kunlin (121) reported his results with 11 venous grafts implanted by suture technique after excision of segments of occluded peripheral arteries. There were two failures.

The next decade brought additional reports regarding treatment of segmental occlusion. In 1950 Sternhardt (122) described 12 cases in which he resected the segments and inserted venous grafts measuring 8 to 30 cm. in length. The results in 4 cases were excellent; there was moderate improvement in 3. In the same year Holden described a case of segmental occlusion of the femoral artery which was successfully treated with a 15-cm. segment of saphenous vein (123). Bouchard (124) discussed use of grafts for obliterative disease. Gosennus (125) reported on nine venous grafts of which only two were known successes. Graft therapy was reported by Fontaine (109, 126) in 28 patients with arterial occlusive disease. Although 15 patients showed excellent results, a significant number, while improved, had no demonstrab[illegible]

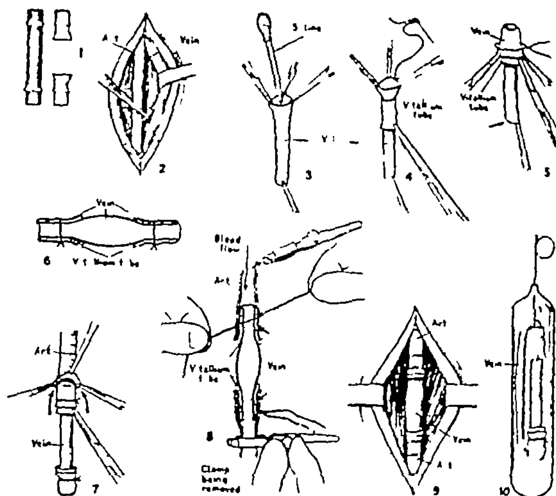


FIG. 147. Method described by Blakemore and Lord for preparation of venous graft for non-suture technique employing flared tubes of vitallium metal. The authors suggested storage of such grafts by refrigeration after quick freezing them in sealed glass ampules. (Courtesy of A. H. Blakemore and J. W. Lord, Jr. Ann. Surg. 121:435, 1945.)

ency of the graft and still others failed. In 1951 Bitzner (127) again wrote concerning the use of venous graft, this time reporting on 90 replacement at the clinic at Freiburg. Many favorable results were li ted, but *most of the patients had been treated for aneurysm.*

Kunlin made a significant contribution to technique with his description of an effective procedure of implantation in segmental occlusive disease in 1951 (128). Into 17 patients with extensive pathology he inserted venous grafts up to 52 cm. in length as a by-pass with end-to-side anastomosis immediately above and below the occluded segment. The advantages of this procedure were 1) retention of collateral circulation, 2) availability of a relatively normal site in the arterial wall for anastomosis, 3) a larger aperture in the artery because the beveled end of the graft offered greater area than a cross-section, 4) less dissection and operating time. Kunlin observed ten early successes but he also had three late failures up to 18 months following operation. These results attest to the highly difficult nature of occlusive disease in respect to successful *treatment with graft.*

In 1951 Freeman (129) tried the inlay technique in the management of aneurysm of the abdominal aorta but decided that veins were not the most suitable material for use in the aorta. During the next year additional reports appeared concerning the use of venous graft in peripheral arteries. Dimtza (130) employed 10 grafts with good result in only 4 of them. Malan (131) had 4 cases, but 2 of them failed early because of hemorrhage or thrombosis. Borghetti and Pezzi (132) reported success with an 8-cm. segment of superficial femoral vein used for an aneurysm of the common iliac artery. Dalahe and Bergau l (133) used a 7-cm. saphenous graft for segmental ... lusion and

as Borghetti and Porzi had done, they heparinized the patient postoperatively

In 1952 and 1953 several reports appeared in the American literature Julian and Dye and their associates (134–136) employed 31 grafts for occlusive disease but approximately half of them failed. DeCamp (137) voiced a preference for venous grafts in peripheral arteries but did not cite personal statistics. Conley (138) implanted saphenous or superficial femoral vein into the carotid arteries of 11 patients with malignant disease requiring radical excisional therapy There was a significant number of failures, but this might have been anticipated in view of the general condition of some of the patients previous irradiation of the local area, and presence of infection in ulcerated tumors. In 1953 and 1954 several reports by Cooke (139) Hughes (140) Jahnke and Seeley (141) indicated their preference for venous grafts instead of arterial homografts in the treatment of traumatic arterial lesions occurring during the Korean War In a small series of cases venous grafts were employed in most of the patients and the follow up time was brief

Ejrup and Hiertonn in 1954 (142) reported use of saphenous vein in three patients with femoral arterial occlusion One graft failed 4 months after implantation In the same year Fontaine (143) published an excellent review of the historical aspects of the use of venous grafts, and described in detail approximately 60 cases from his clinic in Strasbourg Of 48 vein grafts only 27 per cent remained patent. Also in 1954 Mentha (144) reported success with a venous graft used for traumatic dissecting aneurysm of the brachial artery In 1955 Fontaine and his colleagues (145) reported a good result in a 5-year follow-up of a venous graft used for an acute thrombosis of the axillary artery complicating a dislocation of the shoulder In the same year Pilven (146) had success with a short segment of cephalic vein used for rupture of axillary artery associated with a fracture of the humerus. Venner (1056) (147) also employed the cephalic vein for traumatic laceration of an axillary artery Julian (1955) (148) and Dye (1956) (149) and their colleagues described the use of saphenous vein for popliteal aneurysm in five patients and also gave a long follow-up report on their cases of occlusive disease treated with venous grafts, both autogenous and homologous There had been two late closures in patients with autografts. Of special interest was the occurrence of a localized aneurysm in one of the venous grafts



Fig 148 *Above* Fresh autogenous popliteal vein graft used to bridge 8-cm gap in popliteal artery after excision of arteriosclerotic aneurysm *Below* Excised arteriosclerotic aneurysm showing groove formed by the popliteal vein The latter was employed as a graft in the popliteal artery

The author has had success in replacing excised popliteal aneurysms with segments of ipsolateral popliteal vein. (Fig 148.) Subsequent edema has been transient in most patients. In two reports Murphy and Aust (1955 1957) (150) expressed satisfaction with results of venous grafts but noted as did others, that they were less successful in the treatment of occlusive disease. A significant analysis was made by Shaw (1955) (151) in which he compared the results in occlusive disease with autogenous vein and homologous artery In this series of cases from Massachusetts General Hospital, there was better functional success with homologous arterial grafts especially when long segments were bridged In 1955 Rob (152) and Eastcott (153) in presenting an exhaustive review of arterial reconstruction, stated "unfortuntely many of these advantages which have appeared so sound in theory have not been confirmed in practice, particularly as regards the use of autogenous vein grafts to bridge gaps in arteries.

In 1957 Lord and Stone (153A) and Nyhus and Harkins (153B) reported very satisfactory function with autogenous vein grafts. By contrast, Pratt (1958) (153C) observed only two out of 26 grafts patent after three years.

With the exception of the cases reported by Kunlin, most grafting procedures involved excision of the occluded segment rather than the use of a by-pass graft. Warren (1954–1956) (154–155) and Hoye (1956) (156) emphasized the high number of failures observed in patients with occlusive disease, regardless of the type of graft or technique of implantation. Recent reports by Linton (157–159), Crawford (160–162) and their associates and by Deterling (163, 164) have indicated a preference for the by-pass technique in occlusive disease and suggest that better results have been achieved with arterial homografts in these cases. There is little doubt that of equal importance in dealing with disease are the proper selections of case and site of implantation, as well as adherence to meticulous surgical technique.

Use of fresh autogenous venous grafts in the venous system itself has been uncommon. In general, they have been used to bridge gaps in the portal or splenic veins during performance of a portacaval shunt for portal hypertension (Rousselot, 1952) (165). Segments of vein have been used in the superior vena cava for relief of obstruction but with only fair success (Scannell and Shaw, 1954) (166).

## HOMOLOGOUS BLOOD VESSELS

### Transplantation of Fresh Artery

#### *Experimental Studies*

In 1903 Höpfner (2) achieved the first successful transplantation of fresh homologous artery. A segment of femoral artery was transplanted into the carotid artery of another dog employing a non-suture technique. Several years later, Ward (1907, 1908) (167) successfully transplanted fresh grafts of abdominal aorta in dogs. In one specimen examined at 70 days, the muscle and connective tissue cells were described as well preserved, although there appeared to be some decrease in the elastic tissue. In 1908 Capelle (1) reported functional success with fresh homologous arterial graft, but noted degenerative changes at 6 weeks. In 1909 Stich and Zoeppritz (31) described fresh carotid homograft functioning in dogs for 4, 5 and 11 days. They reported little or no gross or microscopic alteration. In the same year Borst and Enderlen (5) described the microscopic appearance of a fresh carotid homograft functioning for 22 days in a dog. These authors noted definite signs of tissue destruction as evidenced by poor nuclear staining, breakdown of elastic fibers, swelling of collagen fibers in the adventitia, and an infiltration by leukocytes. The presence of new elastic fibrils was reported as was a flat mural thrombus. In a 29-day fresh carotid homograft in a goat, the authors described a new intimal zone containing connective tissue, muscle cells and elastic fibers. Their over-all results with homografts were relatively poor, and they believed that drying of the vessels prior to implantation might have been a factor.

Yamanouchi (1911) (9) included in his very comprehensive study of grafts a series of fresh homologous arterial transplants in dogs. In aortic segments implanted in the carotid artery for 10 and 30 days there was fragmentation of elastic fibers and little or no staining of nuclei of cells in the media. Leukocytic infiltration of the adventitia was present. A new intimal layer had developed which was thicker near the suture lines and contained new fibroblasts and elastic fibrils. A similar histologic picture was observed when two carotid homografts functioned in the carotid arteries for 27 and 21 days.

Villard (1910, 1911) (8, 168) observed no significant change at 11 days in an iliac homograft transplanted into the carotid artery of a dog. However, in a 62-day carotid homograft in the carotid artery, Villard noted the disappearance of smooth muscle cells and some changes in elastic fibers. He emphasized the appearance of new fibroblasts in the adventitia and intima and believed that new smooth muscle cells had developed in the new intimal layer. In 1912 Ingebrigtsen (169) studied 8 carotid homograft functioning in the carotid arteries of cats for 3 months. Although he had hoped to demonstrate a different pattern of degeneration based on the presence or absence of isoagglutinins in the blood of these animals, he found no significant variation. There was increased fibrous thickening of the intima and adventitia. The media revealed no significant alteration in elastic fibers but there was great variation, ranging from normal to completely destroyed, in the appearance of the smooth muscle cells. Cat tubes

(1913) (11) also observed intimal thickening and destruction of smooth muscle cells in a carotid graft in the carotid artery at 63 days. He described degenerative changes in the elastic fibers as well. In 1917 Goodman (55) also remarked on the fragmentation of elastic fibers and adventitial thickening from new fibroblasts which he observed in a carotid homograft in a carotid artery at 21 days.

The practical difficulties involved in the clinical use of fresh arterial homografts undoubtedly contributed to the lack of interest in them before sound methods of arterial preservation were devised. Then studies were mainly of long term changes in this fresh material compared to that preserved by various methods and for increasing periods. In 1950 Coleman, Deterling and Parshley (170) implanted fresh homografts of abdominal aorta in dogs and study up to 4½ years indicated that the character and degree of degenerative changes did not differ significantly from those observed in homografts preserved by several methods and implanted for a comparable period (fig. 149). It was of interest that the incidence of fragmentation of elastic fibers and the deposition of calcium were greater with increasing periods of function. In general the severity of degeneration was greater in older specimens, but considerable variation existed among animals. Whereas microscopic calcification adjacent to suture line was observed in one graft in less than 2 months, there were other specimens observed at 3 years without evidences of such change.

In 1952 Pate and Sawyer (171) in a joint study with Deterling and associates compared the fresh aortic homograft with freeze-dried homografts and heterografts in dogs. The findings suggested that fresh homografts were inferior to dead freeze-dried ones. There was a slightly higher incidence of hemorrhage and a much higher incidence of thrombosis in the fresh grafts. In explanation of the latter finding the theory was advanced that electric potential differences in the wall of fresh grafts might predispose to thrombosis. By contrast the freeze-dried graft was dead and showed no such difference in potential. More detailed studies in support of this view were subsequently published by Pate and Sawyer (1953) (172). Although electric potential may play some role in the formation of mural thrombi there are other important factors as suggested by the relatively high incidence of thrombosis reported by Deterling and Coleman in certain types of quickly frozen dead homografts. Miller and coworkers (1951) (13) studied small caliber homografts. They implanted ten fresh arterial grafts into the femoral artery of dogs and observed a thrombosis rate of only 10 per cent, which was slightly less than that noted in preserved homografts. In their extensive investigations of vessel grafts Harkins and his associates have studied fresh aortic homografts especially in the growing pig, (1953) (22, 23). They observed less growth in the homograft than in the host thoracic aorta. Studies by Fisher and his colleagues (1956) (173) compared fresh and freeze-dried aortic homografts in dogs. As in the studies by Pate and associates more late degeneration of elastic fibers and much more thrombosis of fresh grafts were observed.

The microscopic alterations occurring in fresh aortic homografts following implantation have been depicted in much more detail now that the advantages of modern histologic techniques, and longer periods of observation have been brought to bear. Implantation is almost immediately followed by an inflammatory response of varying degree which is thought to be the result of tissue antigenicity. It is of interest that Ward, Swan, Harkins, and a few others have observed a rare aortic homograft in the dog or pig with little or no histologic alteration following implantation. Most commonly, however, there is a gradual death and disappearance of the cells of the transplanted homograft. These alterations are evident within hours in the endothelial cells, within a few days in the smooth muscle cells, and slightly later in

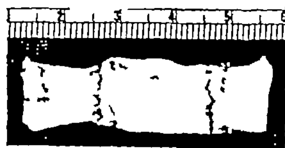



Fig. 149. Fresh canine aortic homograft 20 months after implantation into abdominal aorta. There has been complete endothelization and fibrous union with adjacent aorta. The graft has not thinned nor dilated and no gross calcification is evident.

the fibroblast. The outer portion of the graft is involved to a greater degree than the inner part in the early process. The failure of viable cells in the graft to persist has been established by additional methods such as histochemical assay and tissue culture. Within a few days following implantation there is an outgrowth of fibroblasts from the host tissue surrounding the graft as well as from the intimal zone of the host vessel in the region of the anastomosis. In time the new intimal covering of the homograft, which appears to be derived mainly from the host vessel—although possibly to a lesser extent there are derivations from the circulating blood—is a multilayered fibrocellular zone covered by cells resembling endothelium. The layer is thickest adjacent to the suture lines and in long grafts the thickness in the mid-portion may consist of only a few cells. This process of investment by host cells—predominantly fibroblasts but perhaps also endothelium—takes place concomitantly with the necrosis and disappearance of cells of the graft. Removal of debris is achieved by macrophagic activity.

The rate and distribution of the cellular investment have been studied by various methods. The generation of endothelium and subsequent development of a cellular covering have been studied by silver nitrate staining methods described by Peirce (174) and Bollack (88). The ingrowth of fibroblasts has been quantitated

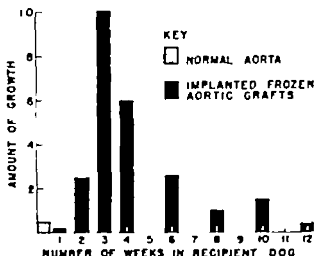



Fig. 150. Amount of fibroblastic growth by tissue culture of implanted frozen aortic homografts following removal from recipient dogs at varying periods of time. (Courtesy of R. A. Deterling, Jr., and associates: Surgery 29: 419, 1951.)



Fig. 151. Photomicrograph of fresh canine aortic homograft implanted 21 months. All cellular elements of the graft have disappeared but occasional fibroblasts from the host have infiltrated the media in some areas. The new intima has decreased in cellularity as has the adventitial zone. (Hematoxylin and eosin stains.) × 67.

by absorption of radioactive phosphorus (175) and by tissue culture (176). Within a few weeks to months there is some capillary ingrowth in the adventitia (177–181). Ingrowth of unmyelinated nerve filaments probably occurs at a later date. Additional studies of the healing process of fresh homologous aorta and carotid artery in dogs have been reported by Benini and Bellinazzo (1953) (182, 183) who isolated some of the grafts from surrounding tissue by tubes of polyethylene. The over-all functional success approached 85 per cent. There has been no conclusive evidence that new smooth muscle cells are produced in the homograft despite descriptions by Villard (168), Nageotte (184, 185) and Hertonn (186, 187). Similarly, there is no proof that new elastic fibers are produced, although some authors have described very fine filaments in the intima, especially adjacent to the anastomosis, which accept elastic stain (Borst, Villard, Yamanouchi, Hertonn). Indeed, Schloss and Shumacker (188) stated of their experiments: "there was no evidence to suggest the formation of elastic fibers in the internal fibrous layer . . . ."

The heavy growth of fibroblasts in and about all types of homografts appears to reach a peak between 1 and 3 months and then according to histologic and tissue culture studies, a slow regression in cellularity begins (fig. 150). By 18 months there is a distinct decrease in cellularity of the new intima and adventitia and the graft itself has long since lost all evidence of cellular integrity. When prepared with hematoxylin and eosin stain, the intimal and adventitial zones show hyalinization and a



FIG 162 Comparison of elastic fibers in fresh control canine aorta (*above*) and in aortic homograft excised after 3 years and 8 months in dog (*below*) Note the condensation of elastic fibers in the homografts with fragmentation and splitting In some areas there appears to be a loss of elastic tissue The graft had been stored one day at 4 C in BSS and serum These changes are found in fresh canine aortic homografts and those preserved by various methods (Verhoeff stain ) ×1150 (Courtesy of C C Coleman Jr and associates Surgery 37 64 1055 )

the fibroblast. The outer portion of the graft is involved to a greater degree than the inner part in the early process. The failure of viable cells in the graft to persist has been established by additional methods such as histochemical assay and tissue culture. Within a few days following implantation there is an outgrowth of fibroblasts from the host tissue surrounding the graft as well as from the intimal zone of the host vessel in the region of the anastomosis. In time the new intimal covering of the homograft, which appears to be derived mainly from the host vessel—although possibly to a lesser extent there are derivations from the circulating blood—is a multilayered fibrocellular zone covered by cells resembling endothelium. The layer is thickest adjacent to the suture lines, and in long grafts the thickness in the mid-portion may consist of only a few cells. This process of investment by host cells—predominantly fibroblasts but perhaps also endothelium—takes place concomitantly with the necrosis and disappearance of cells of the graft. Removal of debris is achieved by macrophagic activity.

The rate and distribution of the cellular investment have been studied by various methods. The generation of endothelium and subsequent development of a cellular covering have been studied by silver nitrate staining methods described by Peirce (174) and Bollack (88). The ingrowth of fibroblasts has been quantitated

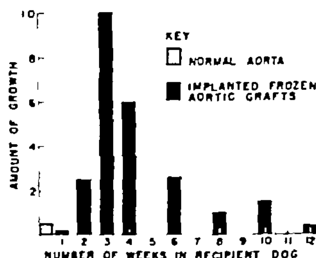



Fig. 150. Amount of fibroblastic growth by tissue culture of implanted frozen aortic homografts following removal from recipient dogs at varying periods of time. (Courtesy of R. A. Deterling, Jr., and a. soc. tes. Surgery 29: 419, 1951.)



Fig. 151. Photomicrograph of fresh canine aortic homograft implanted 21 months. All cellular elements of the graft have disappeared but occasional fibroblasts from the host have infiltrated the media in some areas. The new intima has decreased in cellularity as has the adventitial zone. (Hematoxylin and eosin stains.) × 67

by absorption of radioactive phosphorus (1...) and by tissue culture (176). Within a few weeks to months there is some capillary ingrowth in the adventitia (177–181). Ingrowth of unmyelinated nerve filaments probably occurs at a later date. Additional studies of the healing process of fresh homologous aorta and carotid artery in dogs have been reported by Bec... and Bellinazzo (1953) (182, 183), who isolated some of the grafts from surrounding tissue by tubes of polyethylene. The over-all functional success approached 80 per cent. There has been no conclusive evidence that new smooth muscle cells are produced in the homograft despite descriptions by Villard (168), Nageotte (1... 186) and Hertonn (186, 187). Similarly there is no proof that new elastic fibers are produced, although some authors have described very fine filaments in the intima, especially adjacent to the anastomoses, which accept elastic stain (Borst, Villard, Yamanouchi, Hertonn). Indeed, Schloss and Shumacker (188) stated of their experiments "there was no evidence to suggest the formation of elastic fibers in the internal fibrous layer ..."

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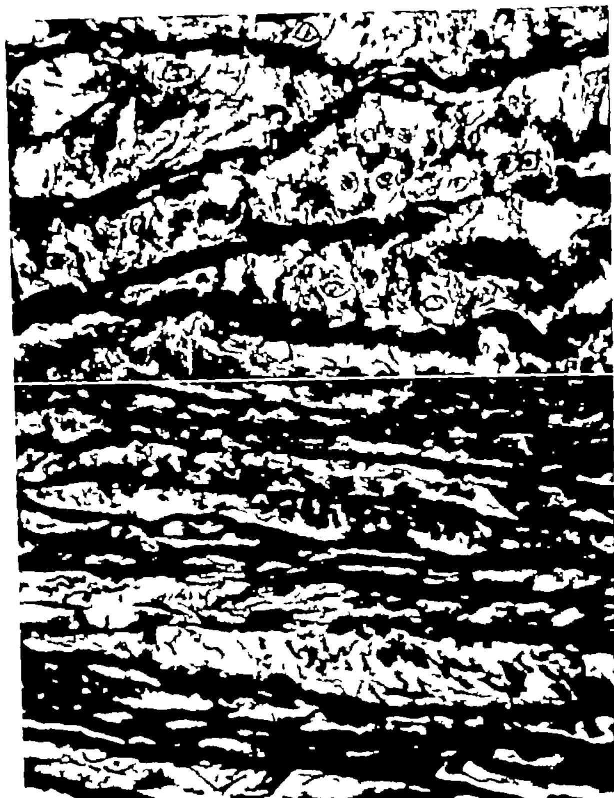


FIG 162 Comparison of elastic fibers in fresh control canine aorta (*above*) and in aortic homograft excised after 3 years and 8 months in dog (*below*) Note the condensation of elastic fibers in the homografts with fragmentation and splitting In some areas there appears to be a loss of elastic tissue The graft had been stored one day at 4 C in BSS and serum These changes are found in fresh canine aortic homografts and those preserved by various methods (Verhoeff stain ) ×1150 (Courtesy of C C Coleman Jr and associates Surgery 37 64 1055 )

fibers and slight calcification Harkin s group investigated fresh homologous venous grafts in the thoracic aorta of growing pigs (22 23) The authors found cellular degeneration and loss of elastic tissue as well as a variable response to growth In some instances the grafts were dilated whereas in others there was somewhat less increase in diameter than in the host aorta.

*Clinical Use*

The greater availability of fresh homologous vein is its only major advantage over arterial tissue, and even then it is an advantage which has its limitation. If one considered varicose veins from the operating room as suitable it is conceivable that material might be obtained from one patient for use in another without significant delay It is unlikely however that anyone with a critical opinion of vessel grafting would advocate the use of varicose veins or the transfer of fresh homologous material without proper screening of it by laboratory tests. If one hoped for a normal vein the problem of procurement is probably as great as that of obtaining fresh homologous arterial grafts. The few studies of fresh homologous vein in animals suggest that this material is at least potentially inferior to fresh homologous artery Insofar as the medical literature indicates there has been little or no clinical use of fresh homologous vein

## PRESERVATION OF BLOOD VESSELS

### Historical Background

Even the earliest investigators recognized that delayed implantation of a blood vessel graft might be both practical and necessary They also appreciated that without proper treatment grafts not immediately implanted might develop undesirable alterations and be less successful functionally (Borst and Enderlen) (5)

In 1906 Carrel (39) began his pioneering work on preservation of blood vessels, and during the subsequent 6 years he and his colleague Guthrie performed experiments estimating the effects of high and low temperatures, electrolyte solutions and devitalizing chemical agents on functional results. Some of the conclusions were not entirely correct and they exerted an influence on recommendations for the establishment of clinical arterial banks four decades later In 1945 a plan for cold storage of venous homografts aimed primarily at military use was proposed by Blakemore and Lord (67 68) Such banks were not developed, however

In 1948 Gross and his associates (190) revived interest in preserved arteries because of a specific need in the surgical correction of certain patients with coarctation of the aorta These authors explored the use of formalin and of quick freezing as well as refrigeration in serum or in an electrolyte solution containing homologous serum. Because of the apparent dependence of satisfactory function upon viability of cells in the graft at the time of implantation, as was demonstrated by tissue culture methods a preference for refrigeration at 4°C in nutrient electrolyte solution was established. Only after several years did experience with non viable quickly frozen and freeze-dried vessels clearly prove that viability of cells in the graft at time of use was not essential to function (Hufnagle (191–194) Deterling (195 196) Pate and Sawyer *et al* Somewhat later restrictions on human donor material were eased in regard to time after death medical conditions at time of death and sterility of grafts. Despite these modifications which increased the number of vessels considered satisfactory for banks the need for homografts generally exceeded the supply and interest in synthetic replacements has increased considerably In most large hospitals synthetic grafts are now used exclusively despite former reliance on preserved homografts

### Procurement of Homologous Human Arteries

Understandably when Gross and associates published their initial conclusions regarding preservation of human material the selection of material was subjected to strict criteria. Many of the restrictions were on the basis of experimental observation while others were the result of assumption and caution. A review of these criteria is pertinent.

*Time after Death*

Initially it was considered that the safe period during which vessels might be obtained for a bank was the first six hours following the death of the donor This recommendation arose partly from the fact that vessels wrapped in saline soaked gauze and refrigerated at 4°C appeared to be less successful as grafts if used after six



FIG 153 *Above* Canine aortic homograft which had been irradiated with a Capacitron prior to implantation (Electronized Chemicals Corp Brooklyn New York) Following brief storage in Hanks solution and 10 per cent homologous serum the graft was implanted into the abdominal aorta for 6 months Note the extensive calcifications *Below* Photomicrograph of another homograft which had been irradiated with 1 6 million rep prior to implantation The structure of the graft has been significantly altered by extensive calcification The new fibrocellular intima thickened adventitial zone and lack of cellularity in the graft are the usual histologic changes observed in aortic homografts (Hematoxylin and eosin stains) × 45

the effects of a malignant tumor or leukemia. Since then, many groups have made use of tissue from comparable donors but have employed some devitalizing agent for sterilization Some have avoided the use of vessels from patients having leukemia believing that cellular infiltrations in the graft wall may weaken the vessel (Keefer *et al.*)

In summary now that the initial restrictions placed on vessel graft material have been eased most banks obtain tissue without sterile precautions and without regard to specific age or cause of death. The gross condition of the vessel has become the major index of acceptability In general, an effort is made to obtain the material shortly after death but periods up to 12 hours postmortem seem acceptable provided the body is quickly refrigerated after death. The processes of sterilization and of storage at very low temperatures or in a vacuum or chemical solution have rendered the graft non-viable, yet very satisfactory function may be observed—in contrast to the belief of early investigators of this subject.

## Methods of Preservation

### *Electrolyte Solutions*

Beginning in 1906 Carrel stored sterile dog carotid and cat aortic vessels in Locke s solution or physiologic saline solution at just above freezing temperatures for a few days prior to implantation Success with this material encouraged him to try other methods of preservation The less gratifying results of chemical agents or of boiling led him to believe that residual viability was the secret of success. Similarly, the early experience of Gross and his colleagues (1948–1950) (190 203 204) brought them to the same conclusions which they substantiated by citing better functional results with grafts stored for only 6 weeks in an electrolyte solution containing 10 per cent homologous serum than with quickly frozen grafts. By tissue culture viable fibroblasts were produced from the former grafts whereas none was demonstrated in the latter Further support for the use of viable homografts was gained from the experiments of Swan and associates and others (205–209)

The solutions used by early workers were usually physiologic saline or Locke s solution. Carrel tried in addition serum, defibrinated blood Vaseline, and simply humid air He assigned the success of his experiments with these various agents to the state of 'latent life which they maintained. He noted that arteries so treated showed little or no degenerative changes for relatively long periods of time after transplantation in contrast to the rapid alterations observed in devitalized arteries. It is interesting to note that Carrel recorded the same type of degenerative changes in preserved viable arteries as he had seen in fresh arterial homografts. His periods of observation were as long as 600 days an unusual achievement for that era After such a period of time the artery revealed no residual smooth muscle cells but the elastic fibers remained. There was a significant increase in fibrous tissue in the intima with some nuclei resembling those of muscle cells New elastic fibers were described in the intima.

Bode and Fabian (1910) (210) repeated Carrel s

not favor very low temperature ranges. As already mentioned, several later workers considered temperatures above 4 or 6°C optimal for maintenance of viable cells. Much interesting and basic study of the effects of very low temperatures on plant and animal tissue was reported by Luyet, Gehenio and their associates (237). They demonstrated that certain resistant cells and tissues could be vitrified by brief immersion in a liquefied gas (nitrogen, oxygen, air) at a

FIG 155 Method originally employed by first community blood vessel bank was modelled after that recommended by Gross and his associates. Seitz filtration apparatus for filling storage bottles with sterile buffered salt solution (Hanks). See continuation in next illustration

FIG. 155. (continued) Steps in the preparation of human aortic grafts. (Courtesy of E. B. C. Keefer and associates. J. A. M. A. 145: 888, 1951.) See continuation in next illustration

FIG 155 (continued) *Above* Graft from New York Vessel Bank at time of use in first patient April 6, 1950. *Below* Graft in thoracic aorta of this 17 year-old male after excision of long coarcted segment

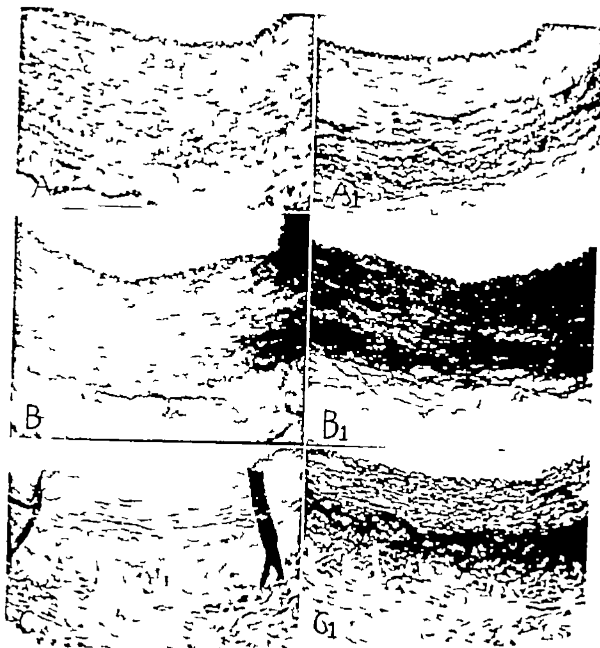



Fig. 154. Photomicrographs of fresh canine aortic tissue and that preserved at 4 C. in Simms' X4 solution and 10 per cent homologous serum. (Hematoxylin and eosin, Verhoeff stain.) ×45.

A. Normal fresh canine aorta showing distribution of cellular elements throughout the wall. The elastic fibers form an orderly and delicate framework, primarily in the media. B. After storage for 76 days at 4 C. in buffered electrolyte solution and 10 per cent homologous serum there has been a disappearance of some of the endothelial cells. Thickness is evident in the smooth muscle and fibroblastic cells. The elastic fibers are slightly more condensed. C. After storage for 5 years there is complete disappearance of cellular elements and the elastic and fibers have become thickened and more deeply stained.

described the methods employed in establishing the first community vessel bank. The storage medium was Hanks' solution with 10 per cent homologous serum as well as antibiotic drugs (Fig. 1 ). Grafts were dispensed to approved hospitals throughout New York City. The method of storage was improved periodically and the bank continued to function with the financial support of the New York Heart Association until July 1958. A community bank has been described by Moore (19 ) (29 A).

*Low Temperature*

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FIG 156 Photomicrograph of canine aortic homograft which had been rapidly frozen in liquid air and stored for 73 days at −70°C before implantation When examined 3½ years later the graft wall revealed areas in which complete rupture of the elastic framework had occurred with subsequent fibrous healing (Verhoeff stain) ×50 (Courtesy of C C Coleman Jr and associates Surgery 37:64 1955)

surgeon (fig 156) Eastcott later employed a two-phase method of freezing whereby the tube containing the graft was transferred from the liquefied gas at 15 seconds to a mixture of dry ice and alcohol or acetone for 20 minutes or so before placing the graft in the storage chamber Later Hufnagel abandoned the use of liquefied gas since he considered the dry ice mixture quite suitable for the freezing phase Deterling and Bhonslay (230) have implanted aortic homografts into dogs after quick freezing at −73°C and storage at −70°C for longer than 5 years Excellent function was observed following implantation for over two years.

Investigation by others has served to establish quick freezing as a satisfactory method of preparation and storage of blood vessels. In 1950 Shumacker and his coworkers (240) studied quickly frozen homografts of vein in the thoracic aorta of dogs and found satisfactory function during a brief period of observation The wall of the graft thickened by 77 days, but no undue dilation was recorded In 1953 Brunnen (241) presented a favorable review of his use of quickly frozen grafts in dogs. In the same year Kremer (242) advocated freezing the graft at −78°C in a nutrient solution. Similar handling of the graft was reported by French and Italian workers. No significant difference in functional success or in ultimate architectural fate has been achieved by these variations in method. In early studies Gross and Hufnagel placed the graft in a tube filled with helium or other inert gases in an effort to effect even more rapid and homogeneous cooling of the tissue. No significant advantage was observed over the results with freezing in air In 1954 Julian and his associates (243) performed experiments with quickly frozen grafts and obtained satisfactory results. Hardin (1954) (244) had good results with this type of aortic homograft in the thoracic aorta of dogs The grafts had been stored for 7 to 120 days at a temperature of −30°C Warwick Brown (1954) (245) found the physical properties of rabbit aorta and cava preserved by freezing methods. Nicks (1955) (246) and Cianciarulo (1957) (246A) confirmed the earlier work by Deterling and associates by reporting an increased rate of failure by thrombosis in grafts if the storage temperature rose

Clinical experience with quickly frozen arterial homograft has now been sufficient to constitute further confirmation of the effectiveness of this type of replacement (Gross, Hufnagel Rob Eastcott (247) Brunnen Hardin Sharp (248) Sussmann (249) and others)

*Freeze-drying (Lyophilization)*

The technical development of freeze-drying as a means to preserve organic matter is generally credited to the independent work of Shackell (1909) (250) and of Elser Thomas and Steffen

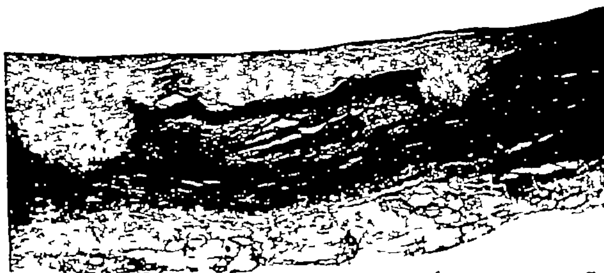


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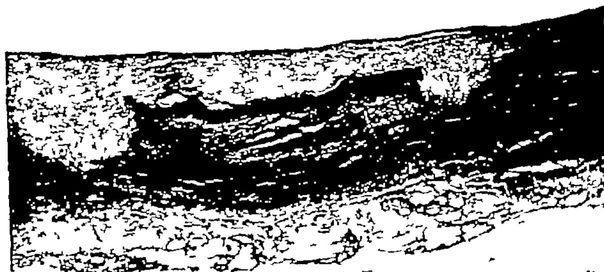


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Hipp and associate (266) Sauvage and associates (267) Scott and associates (268) Taber (269) Hallén Evstiugov and associates (270) Sarlin and Conze (271) Flewett and associates (271A) Heborer and Giessler (271B) Lund and Lund (255A) Gardner and Leemans (271C) Sunderland and associates (255B) and others] In most instances the bank has been associated with a university hospital and its procedures have been based on experience derived from preliminary experimental work with freeze-dried vessels. It is likely that this preservative technique is currently the most widely used since it permits long safe storage of material and easy transportation to other hospitals. This is of considerable importance in certain areas in Europe and Latin America.

*Chemical Agents*

*Formalin* Many authors have investigated the effectiveness of arterial grafts fixed in various percentages of formalin. In 1907 Guthrie (44 45) reported on the use of 2.5 per cent formalin for preservation of vessels to be implanted experimentally as homo- and heterografts. In one instance, cat aorta was used successfully in the carotid artery of a dog. During the years 1907–1909 Levin and Larkin (272 273) studied aortic homografts in the thoracic and abdominal aorta implanted after storage in 4 per cent formalin. In one specimen examined at nine days after transplantation there was necrosis of the inner half of the vessel wall and moderate degeneration in the outer portion. The elastic fibers were intact but stained poorly In an 11-day graft the entire wall appeared homogeneous and without cellular definition. A mural thrombus was observed in a 10-day specimen and an occluding thrombus was present in a 10-week graft. The latter also had areas of calcific deposition and damaged elastic fibers. Prior to implantation the grafts appeared white and rather stiff but had a normal appearance microscopically Some condensation of tissue was noted after periods of storage, probably from loss of tissue and intracellular fluid. Bode and Fabian (1910) (210) reported a formalinized femoral arterial segment functioning for 6 days in the carotid artery of a dog. In 1911 Yamanouchi (9) described a successful graft of aorta placed in the carotid artery of a dog for 24 days. He then found fragmentation and partial disappearance of elastic fibers as well as absence of nuclear staining in the graft There was a thickening of the intima and what he described as new elastic fibrils. Less functional success was enjoyed by Fici and Speciale (1924) (274) who reported 6 failures from thrombosis occurring from 3 days to 3 months.

Twenty-five years passed before the next report on formalinized arterial grafts appeared. In 1949 Peirce and his coworkers (275) claimed good results with homografts in the abdominal aorta of 10 dogs, after the grafts had been stored in 4 per cent formalin. Satisfactory function for 2 days to 9 months was recorded in all but one animal in which there was a thrombus at the site of the proximal clamp Shumacker's group (1950 1951 1952) (240 276 277) called attention to a significant incidence of early calcification in homografts which had been preserved in formalin In France d Allaines (226) reported functional success in 3 dogs (1950) and Oudot (1951) (278 279) also reported on formalinized homo- and heterografts in the thoracic aorta of dogs While reasonable functional success was noted there was moderate early degeneration with fragmentation of elastic fibers. In 1951 Letac (280) successfully employed a homograft of external iliac artery preserved in 4 per cent formalin, as a replacement in a patient with an aneurysm of the subclavian artery Ohara and Deterling (1952) observed satisfactory function with canine aortic homografts preserved in 4 per cent formalin, but the follow-up period was brief No histologic degenerative changes were noted during storage for as long as 3 months. Following implantation of grafts stored for 6 months or less, there was a moderately severe inflammatory reaction followed by loss of cellular detail in the graft In time degeneration of elastic fibers and deposition of calcium salts were observed frequently

Brunnen (1953) (241) presented encouraging results with formalinized homografts in dogs. Leger (1953) (281) was favorably impressed with formalin preservation of homografts and described the technique developed by the Dutch surgeon Nuboer In 1954 and 1955 reports by Nuboer (282 283) indicated satisfactory experimental and clinical use of arterial homografts preserved in 4 per cent formalin Additional details of the studies were presented by Moeys (1954) (284) and a case was reported with full particulars by Van Weel (285 286) A man with an adherent cyst developed a rent in the abdominal aorta during removal of the cyst. Th

(251) In 1943 Weiss (252) applied the technique of freeze-drying to arterial segments which were used as conduits for regenerating peripheral nerve tissue in rats. He observed less tissue reaction to these than to fresh autologous arterial segments employed in similar fashion. The earliest report of freeze-dried artery used for vessel replacement was by Marrangoni and Ceceluni in 1951 (253). Among the 11 femoral and 9 aortic homografts studied for 3 months in dogs there was no thrombosis but hemorrhage occurred in 2 of each group. Subsequent studies continued at the Naval Medical Research Institute by Pate and Sawyer in conjunction with research performed at Columbia University by Deterling and his associates, served to define certain characteristics of the freeze-dried canine aorta (1952). Following preparation in a modified Stokes lyophilizing unit these segments were stored in a vacuum at room temperature until used. During many months of storage there was no evidence of deterioration.

The gross appearance of a freeze-dried vessel belies the very satisfactory manner in which it functions. With water content of the vessel reduced to between 1 and 5 per cent the aorta has the consistency of cardboard. If the vessel is flattened the wall cracks at the crease. The length and thickness of the wall are diminished by virtue of the water loss. An efficient and simple apparatus for the freeze-drying of tissue described by Hufnagel and his associates (1953) has served as the model of equipment employed in many vessel banks.

Microscopic study of the dried aorta reveals essentially normal cellular detail except for condensation of the tissue. After reconstitution of the tissue by immersion in saline for 30 minutes or so normal flexibility and elasticity are regained, and the gross and microscopic pictures resemble those of control tissue. Histochemical studies of reconstituted tissue revealed a loss of water soluble materials such as alkaline phosphatase and glycogen. As was anticipated, no cellular growth was demonstrated by tissue culture. However Massé *et al.* (1957) (253A, 253B) demonstrated contractility of reconstituted freeze-dried and thawed quickly frozen canine aorta.

The absence of electric potential difference in the wall indicates that freeze-dried grafts are biophysically dead. Pate and Sawyer pursued the possibility that the minimal degree of mural thrombosis observed in freeze-dried grafts was related to the electric potential of the wall, since fresh homografts which have a positive charge show a significant incidence of mural clotting. The minimal thrombosis of freeze-dried grafts was discussed as well by Fisher (1956) (254). It is possible that the minimal tissue reaction elicited by the freeze-dried graft may account for the difference in mural thrombosis. Sewell and his colleagues (255) performed experiment sensitizing rabbits with extracts of fresh canine aorta. Subsequent implantation of fresh and of freeze-dried canine aortic segments revealed significantly less reaction to the latter material. The authors concluded that the process of freeze-drying in itself or of reconstitution of freeze-dried grafts modified the antigenicity of the replacement.

Of interest is the report of Brown *et al.* (1959) (17A) who found 64 per cent occlusion of freeze-dried autogenous and homologous canine carotid grafts at one year.

Among the features of the freeze-dried graft which might be considered undesirable is the diminished stimulus to fibroblastic response by the host. This constitutes one of the means whereby the body incorporates the foreign material into its tissues and lends additional support to the wall of the graft. Gross and microscopic examinations of freeze-dried grafts in dogs after months of implantation have revealed thinner walls than are observed in fresh grafts or in those preserved in solutions and implanted for a like period of time. The danger of fracture of the wall of the graft by too rapid freezing or by improper freeze-drying has already been mentioned. Lund (255A) and Sunderland (255B) noted slits in the media of freeze-dried graft. Finally, as Eastcott and his coworkers (1954) (256) have pointed out, atheromata in aortic tissue might make the material unsuitable for grafts since the lipid deposits do not desiccate in the same fashion as aortic tissue. Hence selection of human donors should be rather critical.

The development of freeze-drying equipment and its use for a tissue bank have been described many times. The major variation is in the apparatus employed for the drying process (Hyatt and associates (257), Hufnagel (258), Creech and associates (259), Rob (260), and Eastcott (261), Bernhard and Laufman (261), Collins and Foster (262), Hewett, Lehr and associates (263), Gagnon (264), Henrotin, Jordan and associates (265)

Hipp and associate (266) Sauvage and associates (267) Scott and associates (268) Taber (269) Hallén, Evstigov and associates (270) Sarlin and Conze (271) Flewett and associates (271A) Heberer and Giessler (271B) Lund and Lund (255A) Gardner and Leemans (271C) Sunderland and associates (255B) and others]. In most instances the bank has been associated with a university hospital and its procedures have been based on experience derived from preliminary experimental work with freeze-dried vessels. It is likely that this preservative technique is currently the most widely used, since it permits long safe storage of material and easy transportation to other hospitals. This is of considerable importance in certain areas in Europe and Latin America.

*Chemical Agents*

*Formalin.* Many authors have investigated the effectiveness of arterial grafts fixed in various percentages of formalin. In 1907 Guthrie (44 45) reported on the use of 2.5 per cent formalin for preservation of vessels to be implanted experimentally as homo- and heterografts. In one instance cat aorta was used successfully in the carotid artery of a dog. During the years 1907–1909 Levin and Larkin (272 273) studied aortic homografts in the thoracic and abdominal aorta, implanted after storage in 4 per cent formalin. In one specimen examined at nine days after transplantation there was necrosis of the inner half of the vessel wall and moderate degeneration in the outer portion. The elastic fibers were intact but stained poorly. In an 11-day graft the entire wall appeared homogeneous and without cellular definition. A mural thrombus was observed in a 10-day specimen, and an occluding thrombus was present in a 10-week graft. The latter also had areas of calcific deposition and damaged elastic fibers. Prior to implantation the grafts appeared white and rather stiff but had a normal appearance microscopically. Some condensation of tissue was noted after periods of storage probably from loss of tissue and intracellular fluid. Bode and Fabian (1910) (210) reported a formalinized femoral arterial segment functioning for 6 days in the carotid artery of a dog. In 1911 Yamanouchi (0) described a successful graft of aorta placed in the carotid artery of a dog for 24 days. He then found fragmentation and partial disappearance of elastic fibers as well as absence of nuclear staining in the graft. There was a thickening of the intima and what he described as new e… fibrils. Less functional success was enjoye… Fici and Speciale (1924) (274) who report… failures from thrombosis occurring from 3 … to 3 months.

Twenty five years passed before the … report on formalinized arterial grafts appear… In 1949 Peirce and his coworkers (275) claim… good results with homografts in the abdomin… aorta of 10 dogs, after the grafts had been stor… in 4 per cent formalin. Satisfactory function f… 2 days to 9 months was recorded in all but on… animal in which there was a thrombus at th… site of the proximal clamp. Shumacker's group (1950 1951 1952) (240 276 277) called attention to a significant incidence of early calcification in homografts which had been preserved in formalin. In France d'Allaines (220) reported functional success in 3 dogs (1950) and Oudot (1951) (278 279) also reported on formalinized homo- and heterografts in the thoracic aorta of dogs. While reasonable functional success was noted, there was moderate early degeneration with fragmentation of elastic fibers. In 1951 Letac (280) successfully employed a homograft of external iliac artery preserved in 4 per cent formalin, as a replacement in a patient with an aneurysm of the subclavian artery. Ohara and Deterling (1952) observed satisfactory function with canine aortic homografts preserved in 4 per cent formalin, but the follow-up period was brief. No histologic degenerative changes were noted during storage for as long as 3 months. Following implantation of grafts stored for 6 months or less, there was a moderately severe inflammatory reaction followed by loss of cellular detail in the graft. In time degeneration of elastic fibers and deposition of calcium salts were observed frequently.

Brunnen (1953) (241) presented encouraging results with formalinized homografts in dogs. Leger (1953) (281) was favorably impressed with formalin preservation of homografts and described the technique developed by the Dutch surgeon, Nuboer. In 1954 and 1955 reports by Nuboer (282 283) indicated satisfactory experimental and clinical use of arterial homografts preserved in 4 per cent formalin. Additional details of the studies were presented by Moers (1954) (284) and a case was reported with full particulars by Van Weel (285 286). A man with an adherent cyst developed a rent in the abdominal aorta during removal of the cyst. Th…

traumatized segment was excised and replaced by a homograft of aorta which had been preserved 95 days in 4 per cent formalin at room temperature. The graft had been obtained 24 hours postmortem. At the time of the report the graft was functioning well. In 1954 Creech and his coworkers (286, 287) reported on the successful use of a formalin-fixed homograft in the femoral artery of a patient. These authors generally preferred freeze-dried homografts for clinical use however.

In the same year Warwick Brown (245) preserved aorta and vena cava of rabbits by various methods and tested suture-holding properties of the tissue. He found 4 per cent formalin good in that suture-holding power was enhanced with the maintenance of elasticity. Aorta was stronger than cava. Ross (1954) (288) described the use of 4 per cent formalin for sterilization purposes with subsequent quick freezing or freeze-drying for the period of storage. He noted good results in 12 dogs. Also in 1954 Kimoto and his colleagues (289) reported a comparative study of homografts preserved in formalin and in ethyl alcohol. They observed more failures from thrombosis in the group preserved in formalin. Another comparative study was published by Henrotin (1956) (225) in which homografts of canine abdominal aorta and bifurcation were implanted after preservation in Hanks' solution and homologous serum or in 4 per cent formalin. Although better functional success was observed in the formalinized group, there was a greater incidence of calcification. Fine chromic catgut was employed as the suture material.

Henrotin (289A) described successful use of formalin-fixed aortic grafts in two patients. Tenturier and Lhabibi (289B) had good results with experimental trial of aortic grafts preserved in 4 per cent formalin and planned to use this method in a clinical bank in Morocco.

In 1956 Göthman (290) and Hiertonn (291) published a monograph in which was recorded success in 2 dogs with formalin-fixed aortic homografts stored for slightly more than two months before use. Moderately severe inflammatory reaction was noted microscopically. In the same year Lejeune Ledant, Peters and Albert (292) claimed satisfactory results in the thoracic aorta of dogs with grafts preserved in 4 per cent formalin. Like Kimoto, Madden and McCann (1958) (293) reported a comparative study of homografts preserved in formalin and in alcohol. They observed a higher incidence of thrombotic failure in the grafts preserved in 10 per cent formalin but they improved results by adding heparin to the formalin 24 hours prior to implantation. Because of better results and the simplicity of using 70 per cent alcohol these authors favored this over formalin.

Formalin has also been studied as a fixative of venous grafts. In 1907 Guthrie (44, 45) reported on the use of a segment of canine inferior vena cava preserved in 2.5 per cent formalin for 60 days. Prior to implantation in the carotid artery of a dog, this 0.75-cm. graft was treated with dilute ammonia, absolute alcohol and paraffin oil. At the time of the report the graft had functioned well for 23 days. A remarkable follow-up report on this implant was made by Klotz, Permar and Guthrie in 1923 (294). Eleven years and two months following implantation, the dog died of sarcoma of the sternum. The graft was patent but was calcified and had developed a fusiform aneurysm. The wall was mainly connective tissue with a few remnants of degenerated elastic tissue. In 1950 Shumacker and his coworkers (240) reported on a study of caval homografts implanted into the thoracic aorta of dogs after preservation in 10 per cent formalin. Although functional success was observed in 9 dogs the grafts appeared less satisfactory than a comparable group of fresh homologous venous grafts.

*Alcohol.* In 1918 Nageotte and Sencert (181) reported on a carotid segment preserved in alcohol which functioned successfully for 3 months in the carotid artery of another dog. The elastic fibers were unchanged and a new intima had formed. The authors believed new muscle cells had developed from fibroblasts beneath this layer since the original smooth muscle had disappeared. They termed these new cells "*myome de régénération.*" Later Nageotte described the media of this vessel as appearing to be better than that of a living artery. He also described studies of a heterograft preserved in alcohol. In 1928 Hosomi (295) reported a series of homografts preserved in 90 per cent ethyl alcohol and implanted in dogs for 8 to 407 days. The lumen was obliterated by thrombosis in 21 grafts.

More than two decades later in 1950, Paolucci and Tosatti (296) described satisfactory results observed at 9 months in dogs with homografts which had been preserved for 15 days in absolute

ethyl alcohol. In the same year d'Allaines and Oeconomos (220) commented on the good function of a canine aortic homograft which had been preserved in 20 per cent ethanol. In 1952 Ohara and Deterling (16) performed experiments with canine aorta preserved at room temperature in 80 per cent ethyl alcohol. Normal histologic appearance was retained but grossly the arteries were white and stiff. Observed for a period of one year these aortic homografts showed satisfactory results in the abdominal aorta of dogs. Subsequently Bhonslay and Deterling (297) implanted some of the original material which had been stored in ethanol for longer than five years. Good functional results were noted for over one year following implantation in the thoracic aorta of dogs. Histologic alterations occurring after implantation were of the same order as those observed with canine homografts which had been preserved by other methods.

Studies of heterografts preserved in alcohol were reported by Maeta (1953) (cited in 438). Three sheep arteries functioned well in dogs for a year. Warwick Brown (1954) (245) tested suture-holding power of rabbit aorta and vena cava preserved in 75 per cent ethyl alcohol and found it to be doubled. Elasticity was decreased however. In 1954 Sautot and associates (298) reviewed several studies of homografts preserved in alcohol. They cited the experiments of Di Valmaggiore and Tozatti. Five homografts preserved in absolute alcohol were implanted in 5 dogs which were treated with heparin. There were 4 functional successes. Also cited were the satisfactory results of Oeconomos and Hewitt with 2 homografts which had been preserved in 20 per cent alcohol. It was observed that the morphology of such grafts persisted longer during storage and after implantation than did the morphology of those stored in physiologic solutions.

In 1954 Kimoto (289) presented very encouraging results of experimental and clinical use of homografts preserved in ethyl alcohol. Aortic homografts preserved in 70, 75, and 100 per cent ethanol were implanted in 22 dogs. There were only 2 failures from thrombosis. Histologic alterations were described in detail. Satisfactory results were also encountered in a group of 21 human heterografts used in the aortas of dogs after storage in the several concentrations of alcohol. On the basis of these studies the author employed grafts preserved in ethanol in 11 patients. Of 6 homografts in this series 3 failed because of infection. The remaining 5 implants were heterografts from dog or sheep and recent information reveals that aneurysms have developed in several of them. Catolla-Cavalcanti (1956) (299) described the late modifications observed in functioning aortic homografts which had been preserved in absolute ethyl alcohol. In 1958 Madden and his colleague (293) observed good functional results with canine aortic homografts preserved in 70 per cent ethyl alcohol. As a result of his findings he implanted arterial homografts preserved in alcohol in 2 patients suffering from arteriosclerotic segmental occlusion of the iliac artery. Although postoperative thrombosis occurred in the contralateral artery the grafts functioned well. Enerson and Galante (1957) (290A) described moderately good functional results with aortic homografts preserved in 70 per cent alcohol and implanted into the superior vena cava of dogs.

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### Methods of Sterilization

#### *Antibiotic Agents*

The results of the early experiments of Gross and his associates (1948) (190) seemed to confirm the belief of Carrel that functional success of a vascular homologous graft was dependent upon the viability of its cellular elements at the time of implantation. In order to achieve optimal conditions for maintenance of viability of aortic tissue during a period of storage Hanks (211) proposed the use of a physiologic electrolyte solution—a modification of Tyrode's solution with the addition of 10 per cent homologous serum. Despite the use of aseptic technique when securing vessels and storage at 4 to 8°C., growth of bacterial and fungal contaminants occurred in a significant number of specimens. In an

traumatized segment was excised and replaced by a homograft of aorta which had been preserved 65 days in 4 per cent formalin at room temperature. The graft had been obtained 24 hours postmortem. At the time of the report the graft was functioning well. In 1954 Creech and his coworkers (259, 287) reported on the successful use of a formalin-fixed homograft in the femoral artery of a patient. These authors generally preferred freeze-dried homografts for clinical use, however.

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## Methods of Sterilization

### *Antibiotic Agents*

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attempt to reduce this loss of valuable experimental and clinical material penicillin and streptomycin were added to the storage medium. In general concentrations of 1000 units of penicillin per ml. and of 50 units (50 μg.) of streptomycin per ml. were recommended. In such amounts these agents did not significantly inhibit fibroblastic growth as was determined by tissue culture. Parshley demonstrated toxic effects on fibroblastic growth when the concentration of penicillin was 5000 to 10 000 units per ml. or that of streptomycin was 500 to 1000 units per ml. Of the two agents streptomycin was by far the more injurious. Lazzarini (1952) (302) used aureomycin in addition to the other two drugs. On the other hand, Sautot (1952) (303) preferred chloromycetin. Experiments by Deterling and Mann indicated that contaminated aortic tissue obtained from routine autopsies without sterile precautions contained a variety of organisms in a concentration of $1 \times 10^3$ or $1 \times 10^4$ per gram of tissue. Gram-negative organisms usually predominated in abdominal tissue with low concentrations of staphylococci and streptococci also present. It was impossible to sterilize this tissue by antibiotic agents, even when high concentrations were employed after repeated washes of the aortic tissue in sterile saline solution.

On the other hand Lund and Lund (1957) (235A) sterilized autopsy material with various mixtures of penicillin and streptomycin or neomycin and then freeze-dried the aorta. In 21 clinical cases there was no infection noted in these grafts.

*Irradiation*

The first effective sterilization of contaminated aortic tissue was reported by Meeker and Gross (1951) (108 109). These authors with the cooperation of Trump and his colleagues (304) at the Massachusetts Institute of Technology irradiated contaminated aortic tissue in the frozen state. A high voltage cathode ray of approximately two million "rep" (roentgen equivalent physical) emitted by the van de Graaff electron generator was effective for killing the bacteria, fungi, molds and viruses to be expected in autopsy material. With higher doses tissue damage was observed whereas lower doses at times failed to achieve complete sterility. Following irradiation the tissue was stored at low temperatures (below −40 C.) in sealed glass tubes or polyethylene bags. Subsequent work with electronic irradiation was reported by Hu and colleagues (200, 201) who cooperated with Brasch and his associates at the Electronized Chemicals Corporation Brooklyn New York. These workers employed a Capacitron to generate high voltage microsecond discharges of electrons and found the most effective dosage to be between 1.0 and 2.0 million rep. Confirming the work of Meeker and Gross, they observed loss of tensile strength and other evidence of tissue damage with five million "rep." However in contrast to the findings of the Boston group Hu and his coworkers found consistent and moderate calcification in all the canine aortic homografts irradiated with 1.6 million "rep" functioning for a year. In 1953 Brunnen (211) described studies of irradiated material and considered the thrombosis and rupture of graft in his series as complications of irradiation.

*Cobalt*[60] *has been employed as a source of* irradiation for the sterilization of aortic tissue. MaCris and his associates (1954) performed experiments in which graded doses of gamma radiation from 15 000 to 5 million "rep" were administered from a 10 000 curie source. These authors found a dose of 2 million "rep" competent for sterilization without tissue damage. In a 10-month graft there was a small area of calcification, which the authors did not believe resulted from the irradiation. Buchannan and Marrangoni (1955) (305) also reported studies with cobalt[60] and concluded that too long a period of irradiation was required to be as practical as other methods. According to this study a dosage of 50 000 "rep" was sufficient to sterilize some specimens obtained at routine autopsy.

X-ray *irradiation* has been tested by Deterling and Mann. With equipment standard for radiologic therapy aortic tissue subjected to known concentrations of organisms was irradiated. Although sterilizing doses could be delivered, more time was required than was practical. This material so treated was not implanted into animals.

*Chemical Agents*

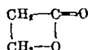
*Prophylactic.* Several authors have described the use of chemical agents to prevent growth of a casual contaminating organism in the preservation of material obtained by aseptic means. Enjalbert and coworkers (1952) (306) employed ortho oxyquinoline sulfate for this purpose

Mortensen, Weed, and Grindlay (1954) (307) found a 1:1000 solution of merthiolate satisfactory.

*Bactericidal.* Following the demonstration by Meeker and Gross that contaminated material could be sterilized and used as vascular grafts with satisfactory functional results, other means than irradiation for effecting sterility were studied. Hufnagel and his associates (1953) rendered contaminated aortic tissue sterile by brief contact with ethylene oxide. This volatile and inflammable fluid had to be used with suitable precautions. Because of the simplicity of this method of sterilization and because the chemical is removed from the tissue during the process of freeze-drying, the use of ethylene oxide became popular. Experimental work by Zech and associates (1954) (89), Ross (1954) (288), and Eade and associates (1956) (308) have confirmed the fact that, if used properly, ethylene oxide is effective in sterilizing contaminated aortic grafts without damaging the tissue. Eade and his associates presented evidence that showed exposure to ethylene oxide for longer than 30 minutes modified the graft, and poor functional results ensued. Two of five such grafts ruptured. Sewell and his colleagues (1954), by exposure of aortic tissue to ethylene oxide for 20 hours at 0°C., damaged it sufficiently so that aneurysm formed in 7 grafts after implantation of the tissue as homografts in 10 dogs. In the area of the aneurysms the elastic fibers were severely damaged or absent.

Several workers have reported varying results of trials of various other chemical agents including ethylene carbonate, ethylene amine, ethylene imine, propylene oxide, trimethyl phosphate, and thioglycollic acid. In general, there have been moderate denaturation of the tissue protein and inflammatory response by host tissue to grafts treated by these agents. In some instances there has been excessive calcification, in others, failures by thrombosis, in still others, thinning and dilation (LoGrippo and coworkers, Eade and others).

Stimulated by observations by Hartman on the action of beta propiolactone, Trafas (309), LoGrippo (310) and their respective associates showed that a one per cent solution was more than adequate to sterilize effectively contaminated aortic tissue with little or no denaturation of proteins. The functional success and ultimate fate of canine homografts of aorta so treated were equivalent to those of controls. Subsequently Szilagyi and associates (1954) (202) reported more detailed observations of the effectiveness of this agent in clinical and experimental trials. Beta propiolactone is an ester of hydroxy-propionic acid and has the formula



In dilute concentrations in water the chemical is unstable, and the lactone ring is capable of opening either at the alkyl or the acyl oxygen bond. It then reacts readily with SH, COOH, OH, phenolic or amino groups, a process which is aided by raising the temperature to 37°C. In the treatment of arterial grafts for human use the tissue is placed in a flask containing

- 0.85 per cent sodium chloride solution, 200 to 600 ml.
- 0.2 molar sodium bicarbonate (1.68 gm. per 100 ml. of saline solution)
- phenol red (5 mg. per 100 ml. of saline solution)

Next is added 10 ml. of 10 per cent BPL per 00 ml. of saline-bicarbonate solution. The BPL solution is prepared by dissolving 0.88 ml. of stock BPL in 10 ml. of cold distilled water (4°C.). After incubation in a water bath at 37°C. for 2 hours the graft is removed aseptically and is washed in sterile phosphate buffer (pH 7.4) in preparation for storage. Although Szilagyi and his colleagues favored storage in Hanks' solution at 4°C., others have found quick freezing equally suitable (Deterling and others). Sunderland *et al.* (255B) found BPL superior to ethylene oxide.

It has already been noted that Ross (1954) (288) employed 4 per cent formalin for sterilization of aortic grafts prior to quick freezing or freeze-drying them for actual storage.

## PRESERVED VASCULAR TISSUE

### Arterial Tissue

#### *Experimental Studies*

In 1906 Carrel began a series of investigations of the storage of vascular tissue which has been the inspiration and model for many subsequent studies. Although most of his efforts were confined to one or two animals in the study of each given type of preparation, one must appreciate the difficulties facing pioneer experimental sur-

geons Not only were facilities meager but without the assistance of anticoagulant drugs antibiotic agents blood replacement and safe angiography the pursuit of a satisfactory program was beset by limitations. Those interested in the field of vessel replacement would do well to read all of Carrel's publications both the English and French ones since in series they constitute progress reports with occasional summaries of his broad investigations

In 1910 Carrel (43) (311) discussed some of his results of the study of canine carotid artery preserved in saline solution Locke's solution humid air Vaseline serum, and defibrinated blood. *He thought these media kept the vessel* in a state of "latent life since there was a slow degeneration following transplantation in contrast to the findings in arteries devitalized by freezing boiling, drying, and other methods. In 2 specimens kept at −3°C for several days before implantation he found complete thromboses at 5 and at 85 days. On the other hand of 3 specimens dried by calcium chloride and reconstituted in Locke's solution prior to use 1 functioned well for 202 days. Microscopic examination revealed a thick adventitia and intima In the latter Carrel described elastic fibrils. The *elastic framework of the graft was still intact* but all the smooth muscle had disappeared. In his longest observation of a preserved artery examined at 600 days Carrel observed a thickened intima with nuclei resembling those of smooth muscle cells and also elastic fibrils In this graft the elastic fibers were preserved but the smooth muscle was absent

In 1910 Borle and Fabian (210) repeated certain experiments by Carrel but most of their grafts thrombosed A few were studied after only a few days and early degeneration was noted. Yamanouchi (1911) (9) had success with *aortic grafts implanted into the carotid or femoral* artery of dogs after storage in Locke's solution for 3 to 9 days at 4 C From his examination of these grafts at 9 24 103 and 143 days he confirmed the observations by Carrel that the degenerative changes occurring in preserved arterial homografts were essentially those noted in fresh homologous transplants after implantation. *There was an investment of the graft* externally by connective tissue derived from the host. The intima was thickened by new cell and Yamanouchi *also described elastic fibril* There was a progressive disappearance of smooth muscle cells from the graft and the elastic fibers *fragmented and later* disappeared in some areas In the 24-day specimen the smooth muscle and elastic fibers appeared to be in remarkably good condition. This apparent acceptance of an arterial homograft by the host has also been noted by Swan and associates (1950) (205 209) Parsons Gerbode and Cox (1952) (10) Sauvage and Harkins (1953) (22 23) and others In 2 other animals Yamanouchi studied functioning aorta which had been kept for 5 to 10 days in sterile water at 4°C prior to implantation. In an 8-day specimen there was marked fibroplasia in the adventitial zone. There was some *fragmentation of the elastic fibers of the graft, and the smooth* muscle stained poorly In an 81-day specimen there was dense connective tissue in both adventitia and the outer media The intima was thickened by fibroblastic growth the smooth muscle was necrotic *and the elastic fibers fragmented* and diminished

Villard and his coworkers (1910–1911) (8, 10) studied a successful carotid arterial transplant to the carotid artery after refrigeration for 19 days. At 48 days the graft was sequestered between new fibrous intima and adventitia. Elastic tissue was intact, but the muscle had *disappeared. In iliac graft studied at 31 days* after transplantation to the carotid artery there was some degeneration of the elastic tissue and no new intima was noted This graft had been stored 67 days in serum at about 4°C Because of the small number of successfully functioning experimental arterial homografts, the evidence of degeneration of the graft after implantation and the problems *posed in considering the establish*ment of clinical vessel banks surgical interest centered in the autogenous venous transplant As has been noted a number of such grafts was used in patients after 1907 and especially by *German military surgeons* during and following World War I

In 1948 Gross and his associates (186) described studies of *preserved arterial homograft* which were sufficiently successful to lead immediately to the establishment of a clinical blood vessel bank. In their initial report (1948 10.0) (190 200 201) the experimental evidence *favored the "viable" graft* and confirmed in a sense Carrel's belief in the importance of maintaining latent life " Since their functional results *with grafts kept at 4 C in Hank* solution and 10 per cent homologous serum were superior t

those kept in formalin or those subjected to freezing, it is understandable that these observations should have formed the basis for criteria recommended for clinical banks. Considerable difficulties in maintaining an adequate supply of grafts were imposed by the supposed necessity of discarding the grafts after only 4 to 6 weeks of storage. Although many workers were convinced at that time that viability of preserved aorta was essential for successful function, additional experience with dead frozen or freeze-dried grafts soon established the suitability of non-viable material for clinical use (Hufnagel, Deterling and associates, Meeker and Gross, and others).

Many informative experimental studies have been made of preserved aortic tissue with regard to the influence of viability on functional results. Weiss (1943) (252) expressed doubts as to the value attributed to viable homologous material and believed that there should be better methods for investigation of the many factors influencing homotransplants. Peirce and his associates (1949, 1952) (312, 174) compared results reported by numerous investigators and concluded that viable grafts produced better functional results. They believed that the living endothelium played some role in preventing early mural thrombosis and might even take part in the healing process. Rehn (1951) (313) also favored viable material for essentially the same reasons. Swan and his coworkers (1950) (205, 206) presented data on canine aortic homografts which showed less degenerative changes in implants which had previously been stored in Ringer's solution for less than 40 days than in those which had been stored longer. These authors observed that viability as determined by tissue culture was not essential for successful results. Deterling and his associates (1951) (230) performed experiments with quickly frozen grafts as well as with those stored a brief time in Simms' X0 solution with serum. While it appeared in the early results that somewhat better function was observed in the latter group, tissue culture viability of the graft at the time of use bore no relationship to incidence of thrombotic failure. In addition, the pattern of degeneration of both types of homograft was the same. Later the authors reaffirmed this observation in the course of studying a series of long term grafts (both fresh and preserved by various methods); again no significant differences were noted grossly or microscopically up to more than 5 years after implantation (1955).

Hiertonn (1952) (187) studied the viability of 38 preserved aortic segments by estimation of tissue respiration after the method of Warburg. No histologic differences were observed on the basis of viability. However, after periods of function as homografts, the non-viable group of segments occasionally showed more degenerative changes than the viable group; therefore Hiertonn favored the viable type. Massé and his coworkers (1952) (217) described good function but continued degeneration of homografts which were non-viable when implanted. They apparently favored efforts to preserve cells during storage and used blood media. They suggested that the nature of blood grouping might influence the quality of preservation. Sawyer and Pate (1953) (314, 315) elaborated on their studies of electric potential of aortic wall, and concluded that perhaps non-viable material could be superior to viable but dying tissue. The potential differences of the latter might serve to attract platelets and hence produce mural thrombi. These authors observed much more thrombosis in fresh canine aortic homografts than in freeze-dried grafts. Rob (1954) (260) suggested that since cells in homologous aortic transplants do not survive, efforts to maintain viability during storage may be completely unnecessary. He recommended the most convenient type of preservation that would not denature protein.

Bellman and his associates (1956) (181) studied incorporation of $S^{35}$ labelled sulfate into the esterified mucopolysaccharides of the aortic wall to discover whether viability was a requisite for good function. By radioautographs of the media of host aorta and graft material a quantitative estimate of uptake was made. The uptake and function appeared to have a relationship to autogenous aortic grafts but not to homografts preserved in nutrient electrolyte solution, in 4 per cent formalin or by freezing. These investigators concluded "that the functional result after transplantation does not depend on the presence of living material in the transplants. . . ." From a review of the observations of many early investigators of homologous vascular transplantation, it appeared as though viability of the transplant did influence the rate of degeneration following implantation as well as the functional result. As more evidence has accumulated, however, it has become clear that viability of a

vascular homograft is not a requisite to satisfactory function. While the rate of degeneration may appear to be slower in grafts with living cellular components at the time of implantation and while the degree of late degeneration may seem to be less in such grafts it has not been conclusively shown that such differences in these grafts are dependent on viability of the implant. While variations in the rate and pattern of degeneration in aortic homografts of any one type are not the result of any one factor An understanding of the immunologic aspects of the host homograft relationship holds the greatest promise of better comprehension of variability in the behavior pattern of vascular grafts [Szilagyi (315A)].

To the determination of this behavior pattern the findings of innumerable studies of the aortic homograft have already contributed a rather consistent picture. It has been shown that immediately following the implantation of a preserved aortic homograft into the aorta of the peripheral artery of the host, an inflammatory response develops at the juxtaposition of the graft and host tissues. The reaction may be severe with an accumulation of polymorphonuclear leukocytes hyperemia and edema or there may be seemingly complete acceptance of the homograft. In most instances the response is mild and persists but a few days in the acute phase During this period there is a fibrinous adherence of the surrounding tissues to the graft grossly as well as a varying amount of free blood around the graft, depending upon extravasation at the anastomoses. With a technically satisfactory suture line there is minimal blood loss because of the early development of a fibrin clot which seals the anastomoses. This may be observed within hours and organization of the wedge-thrombus during the first week or 10 days occurs with the infiltration of fibroblasts from the host tissues and aorta. Also there may be noted small round cell infiltration in the adventitial zone Insofar as the homograft itself is concerned there is an early loss of any remaining endothelium, so that after 48 hours there is no cellular surface in the lumen. The intact internal elastic membrane may be coated with a thin fibrin layer it remains confluent with the thrombin plug at the anastomoses and possibly thickens adjacent to this area Concurrently there is a loss of cellular detail in the graft The nuclei become condensed and pyknotic The smooth muscle cells are most susceptible to deterioration and may begin to disappear within several days As has been mentioned the rate and degree of this degeneration of the cellular elements of the graft vary but there is a pattern in all types of homografts. The inflammatory reaction is less in general, with freeze-dried vessels than with grafts preserved by freezing or in a nutrient solution. On the other hand, a more intense reaction may be observed with aorta preserved in chemical agents, especially formalin.

In ten days fibroblastic activity in the adventitial and intimal zones increases appreciably The latter becomes a thickened multilayered cellular lining with modification of the innermost fibroblasts to a very flattened endothelial-like layer There is evidence from tissue culture studies that endothelial cells progress onto the graft surface from the host aorta. Occasionally small round areas near the mid-portion of the segment remain uncovered for some time and develop a platelet or fibrin clot which ultimately becomes organized. Some authors have claimed that the circulating blood contributes significantly to the formation of the new intima and do not believe this zone to be derived predominantly from elements of the host aorta. Using tissue culture studies Parshley divided the length of an excised homograft into thirds and planted tissue dissected only from the luminal surface In grafts measuring 2.5 to 4.0 cm. in length there was during the first two weeks following implantation, cellular growth only from the sections adjacent to the host aorta and not from the mid third This would suggest that the aortic elements do play the primary role in reconstituting the inner surface of the homograft. The adventitial zone of the graft was invested by fibroblasts and an ingrowth of capillaries MacPherson and Dutrie (27A) deny that the intima is produced from cells of the circulating blood.

By four weeks the blood about the graft is largely broken down and absorbed. Phagocytic activity may be evident The site of the anastomosis is well organized with fibroblastic infiltration. The graft may be completely acellular appearing as a homogeneous hyaline matrix through which the elastic fibers course These become thickened and stain deeply In occasional areas they may split or even fragment. The adventitial zone blends with the original graft and is mainly fibrocellular although there is significant capillary ingrowth in this zone at [illegible]

penetration even into the outer media of the graft. Occasional unmyelinated nerve filaments may be found in the adventitia as well. The intima has thickened still more, mainly from fibroblastic proliferation. Most recent reports omit mention of new elastic fibrils or smooth muscle cells described by some of the early workers, although Hiertonn does describe such structures in his monograph (1952). Weis (1951) (77) and Medvedev (1954) (316) also raise the question of new elastic tissue.

By virtue of fibroblastic activity the graft excised at about one month may appear slightly thicker walled than it was when implanted. Wall thickness may increase even more during the next few months although such thickening is generally less marked with freeze-dried grafts. This difference has been explained by reduction of antigenicity by freeze-drying and subsequent reconstitution.

After six to ten months there is decreased cellularity of the intima and adventitia, and development of scar tissue progresses. Little or no blood supply to the graft develops *via* the intimal surface (McCune and associates) and apparently nutrition of the intima and inner media occurs by permeation from circulating blood. A complete understanding of the vascularization of homografts is yet to be achieved since the data are not consistent. Parsons, Gerbode and Cox (16) found no increased degeneration of homografts after isolating them from surrounding tissue by silver foil for nine months. On the other hand, Kiehn and his associates, by correlating the microscopic picture and the uptake of radioactive phosphate by aortic homografts following implantation demonstrated that the adventitia was the primary source of blood supply. Benemi and Bellinazzo (182, 183) studied implanted homografts by isolating them from surrounding tissue with thin polythene sheets and by interruption of blood flow through the transplants with bipolar ligation. It appeared that the major nutrition came from the adventitial zone. Minimal and often insufficient nutrition of the intima and inner media was derived from the contact with blood elements on the luminal surface. Göthman studied four aortic homografts in dogs which had been functioning for a year. By the microangiographic technique of Bellman he observed a rich supply of new vessels penetrating the adventitia and outer media and so confirmed the observations of McCune and his coworkers (177, 178). Like the latter Göthman observed no vessels entering the graft from the host aorta through the everted ends of the graft or from the lumen. Moderately large new vessels were observed in the scar tissue at the anastomoses, and these did enter the everted ends of the grafts. Göthman did not consider these vessels as recanalizations of vasa vasora, nor was the presence of new vessel penetration into the media proof that the graft could or did retain viability. Edwards *et al.* (1957) (316A) showed that stasis of blood in a graft for 90 *minutes* would not produce a thrombus if the intima was *intact*.

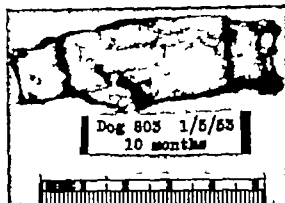



FIG 157 Marked calcification of canine aortic homograft observed 10 months following implantation. The graft had been stored at 4 C for two weeks prior to use

In aortic homografts removed from the abdominal aorta of dogs up to nine years following implantation Deterling and his coworkers have observed progressive thinning and an increased incidence of calcification of the graft, regardless of the type of preservation. However marked calcification may develop in only a few months in some grafts (fig. 157) and be minimal or absent in others examined over nine years following implantation. Of greater significance was the continued degeneration and disappearance of the elastic fibers of the graft (fig. 158). Kremer (1955) (317) also demonstrated loss of elastic tissue by estimations of polysaccharides in the aortic wall. The once luxurious growth of fibroblasts in the adventitia and intima was reduced to a very thin zone of scar with scanty cells. The graft became compressed and at times dilated. No aneurysm was observed in the eighteen dogs of Kremer's study or in four additional dogs observed for seven years following implantation. In 1956 Göthman and Norberg (317A) estimated elastin content of freeze-dried aortic

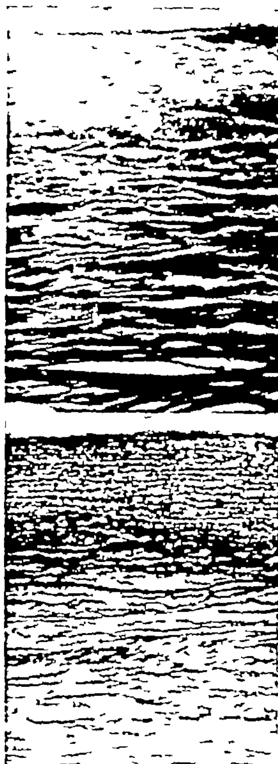


FIG. 158. Photomicrograph of canine aortic homograft. The intimal zone has decreased in thickness and in the mid portion of the graft it is barely recognizable as a layer. The thick fibrous adventitia has become hyalinized scar. The original graft has become thin and appears as a pale granular acellular band. (Hematoxylin and eosin.) The elastic fibers of the graft have fragmented, become condensed in some areas and have disappeared in others. (Verhoeff stain.) × 90

grafts by determination of hydroxyproline in 14 dogs studied up to 270 days. There was no significant difference from that found in host aorta. Excellent observations relating to the pattern of degeneration of homografts in dogs and pigs have been made by Sylhus, Sauvage, Harkins (1953) and their colleagues. Their findings have confirmed those already described, but in addition they reported studies indicating that greater degeneration may occur in the thoracic aorta than in the abdominal aorta (Kanar and coworkers 1951 (318)). Among their investigations were comparative studies of the behavior of different types of graft in the thoracic aorta of the growing pig. The most satisfactory results were observed in aortic autografts, and aortic homografts ranked second in a comparison which included venous auto- and homografts. Kanar's group (1950) (319) demonstrated that composite grafts of autogenous and homologous aorta in the aorta of growing pigs behaved in essentially the same manner as did each type of material implanted independently.

Several studies have dealt with the importance of various physical factors—among them, growth—which might influence the function and ultimate fate of aortic homografts. Johnson studied autogenous aortic and venous grafts in growing pigs and observed a rate of growth equalling that of the adjacent aorta. By contrast, later studies of homologous aortic grafts in the abdominal aorta of growing pigs revealed a limitation in the increase in size of most of the graft. One graft was dilated. Deterling and associates (1949) implanted aortic grafts from puppies into the abdominal aortas of other pups, the grafts first having been preserved in nutrient solution. Growth of the homograft was not observed, although some thinning and dilation were apparent (fig. 159). Shumacker and his coworkers (1951) (270) showed growth of an anastomosis provided interrupted sutures were used with a type of graft capable of growth with the animal. Keefer, Glenn and Dotter (1953) (320) reported on two-year observations of autogenous and of homologous aortic grafts in young dogs. Excellent growth was noted in the autografts, whereas there were slight dilation and calcification of the homografts. Keefer and Glenn (320A) confirmed these findings in homografts at six years. Sauvage and his associates (1953) (?? 23) implanted homografts of aorta into the thoracic aorta of growing pigs. There was little change in the

diameter of the grafts due to a slower rate of growth of the transplant Actually since one must now consider aortic homografts as dead tissue following implantation the differences in the diameter and the length of homografts observed in growing animals result from cicatricial contraction or dilation rather than from growth.

Another physical factor influencing the behavior of homografts is the dimensions of the graft. Early studies suggested that critical matching of diameters of the graft and host aorta was vital in respect to good function (Parkinson and Woodworth (321) Swan and associates, Coleman and associates and others) It now appears that reasonable approximation of the diameter is sufficient. Schmitz and his coworkers (1953) (34) found that in dogs a biterminal disproportion of more than 21 per cent was associated with an increased rate of thrombosis in the graft. These authors developed a plication method for reducing the diameter of grafts, with special reference to autogenous vein. Another consideration is the greater incidence of thrombotic occlusion associated with decreasing the caliber of the graft, as shown by the functional results with canine homografts in the thoracic aorta the abdominal aorta and the femoral or carotid artery (Williamson and Mann (322) Miller and associates (13) Eastcott and Hufnagel, and Deterling and others) Whereas one may expect close to 100 per cent success with a suitable graft and a satisfactory technique in the thoracic aorta the rate falls to perhaps 85 per cent in the abdominal aorta and 70 per cent in the smaller peripheral arteries of dogs.

A few studies of grafts have been concerned with the effect of length upon functional success. Although Schmitz and his associates found less success in dogs as the graft exceeded 5 cm. in length others have observed good function with longer homografts McCune and his coworkers had success with end-to-end and by pass homografts, 22 cm. long, in dogs observed from 3 to 8 months. Adequate function has been noted in several studies of long replacement of the terminal aorta and its branches in dogs and pigs (Dalem, Deterling, Nicks)

Some investigators, working with dogs, have attempted to accelerate degenerative changes in homografts by inducing a state of stress in the animals. Gentsch Waters, and Glenn (1954) (323) administered egg yolk-saline infusions for eight to ten weeks following implantation of

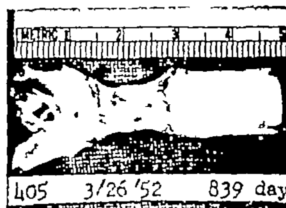



FIG 159 Canine aortic homograft obtained from one puppy and inserted into the abdominal aorta of another puppy of the same age after storage at 4°C in a nutrient solution for two weeks After 830 days the host aorta has grown considerably but the homograft has remained essentially unchanged Slight increase in diameter of the anastomoses can be observed An everting mattress type of suture line with 5-0 braided silk had been employed The graft is well endothelized and there are no remarkable degenerative changes

homografts in the thoracic aorta of dogs. Calcification shortly developed in 50 per cent of the grafts in the stress group as compared to its occurrence in one graft in the control group All of the implants in the stress group revealed a far more extensive infiltration of foam and inflammatory cells than was noted in adjacent host aorta. Creech and coworkers (1955) (36) rendered dogs hypothyroid by radioactive iodine and then fed them a high cholesterol diet after implantation of homografts in the abdominal aorta. In dogs with serum cholesterol levels in excess of 1000 mg. per cent, the host aorta and homograft were equally involved by atherosclerotic lesions at six to eighteen months. In those with lower levels the host aorta showed minimal or no involvement, whereas all homografts were moderately to extensively involved.

Hamovici *et al.* (1958) (189A) found little degeneration in thoracic aortic homografts implanted in the abdominal aorta of dogs given thiouracil and a cholesterol rich diet There was atherosclerosis of the host abdominal aorta but little in the thoracic aorta The authors suggest a possible biologic difference in thoracic and abdominal aorta.

From the many experimental studies of aortic homografts stored in electrolyte solutions with serum there has been more than adequate dem-

on tration of satisfactory function (Peirce Gross Swan Dalem, Deterling Johnson, Colombo McCune Parson Benchini Rot, Henrotin Ma & Harkins Leary Hallen, Hertonn Göthman and others) Although early observations of quickly frozen aortic homografts were discouraging later work established this type as being completely satisfactory (Hufnagel, Deterling Colombo Barberio Brunnen, Leary Nicks and others) Still later experimental evaluation of the freeze-dried homograft established it as very suitable too (Marrangoni, Pate Sawyer Hufnagel Deterling Natellis McKenzie and coworkers (324) Leary Ashburn and coworkers (325) Bryant and Sloan (326), Fisher Bornemann and coworkers (327) Macpherson and Duthie (27A) and others) Several comparative research programs have been conducted in an effort to evaluate both fresh and preserved aortic homografts Peirce Gross and others (1949) (274) were greatly influenced by the poor results they obtained initially with quickly frozen aortic homografts and recommended preservation at 4°C in Hanks solution and 10 per cent serum. Subsequently Gross showed preference clinically for quickly frozen arteries sterilized by the cathode ray In 1949 Deterling and his coworkers initiated a comprehensive study of various types of graft and an evaluation of several methods of preservation Although excellent function was obtained with grafts kept in nutrient solutions, very satisfactory results were also demonstrated with quickly frozen freeze-dried and alcohol preserved canine aortic homografts In a group of transplants subjected to low temperature storage less success was obtained with those kept at a temperature well above −40°C The use of anticoagulants improved the results in dogs but was not considered entirely suitable for routine clinical usage When these authors excised homografts after function up to nine years great individual variation in the degree of degeneration was observed regardless of time of implantation method of preservation and length of prior storage When all data were analyzed a consistent pattern of degeneration of homograft *per se* was evident and the factors just mentioned seemed to have little influence after the first year or two

In 1951 Colombo Teich and Costa (328) published studies of aortic homograft and heterografts preserved in nutrient solution, in formalin, and at low temperatures They demonstrated functional success with all the types of homograft Lanzons and associates (1952) (16) compared autogenous aorta with that stored in Tyrode's solution. Although satisfactory results were obtained with the latter type the autogenous graft was naturally better in function and appearance The extensive experiment by Harkin Sauvage and associates have added confirmation to these observations. Pate Sawyer and coworkers studied freeze-dried arteries extensively using the fresh canine aortic homograft for comparison The latter appeared far less satisfactory with respect to tissue response after implantation, development of thrombosis and degenerative changes during the initial twelve to eighteen months These observations were confirmed later by Fisher Wilde and Engstrom (173) Leary Kelley and Gregg (1953) (231) compared results of canine implants of aortic homografts stored in modified Tyrode's solution, quickly frozen and freeze-dried. These authors obtained their best functional results with freeze-dried grafts too Cianciarulo (210A) found freeze-drying or quick freezing at −72°C to be best

There has been considerable discussion regarding the effect of storage time on ultimate function and fate of aortic homografts. As has been mentioned some of the very early workers expressed an opinion that only short term storage was permissible in order to preserve histologic characteristics and latent life" (Carrel, Bode and Fabian) Gross and his colleagues strengthened this concept by using cultural evidence of fibroblastic growth as an index of the suitable period of storage of aortic tissue A period of about six weeks was considered maximal Swan and coworkers (1950) (205 206) showed less satisfactory results in grafts which had been stored in Ringer's solution and serum longer than this period. Some German investigators favored frequent change of medium and very brief storage Tonelli and Allegra (1956) (329) studied iliac homograft in 8 dogs after storage in saline at 4°C for 7 11 and 20 days. All grafts remained patent but the most striking histologic alterations appeared in the grafts which had been preserved the longest. Hamblin and Lord (1951) (330) believed that the rupture of a clinical aortic homograft occurred because of prolonged storage in a nutrient medium.

When non-viable material was finally considered acceptable there was no scientific [illegible]

for the estimation of safe storage time. Hufnagel believed that frozen grafts could be kept for at least 0 months at −70°C In the opinion of Pate and his associates freeze-dried material stored in vacuum could be kept for at least 2 years. Very few studies have been reported wherein grafts stored for very long periods of time have been used Buchanan and Marrangoni (1955) (305) reported satisfactory results with canine aortic homografts which had been freeze-dried and stored at room temperature in vacuum for as long as 5 years. Recently Bhonslay and Deterling (207) studied a series of dogs in which several types of aortic homografts had been implanted up to 2 years after periods of storage in excess of 5 years. Excellent function was observed in the thoracic aorta of dogs with grafts which had been stored in Simms' X6 and 10 per cent serum at 4 C (and with no change of medium) Excellent results were also obtained with those quickly frozen and stored at −70°C., with those freeze-dried and kept at room temperature in vacuum, and with others kept at room temperature in 80 per cent ethyl alcohol The author has had success in human cases with frozen aortic grafts after storage at −70°C for 18 months. It would therefore appear that early estimates of the maximal period of storage of homografts for satisfactory function were grossly in error

Considerable speculation has appeared in the literature regarding the importance of the elastic framework of the aortic homograft. In the opinion of most workers the integrity of the wall depends greatly upon these fibers, and concern has been expressed for the potential ultimate fate of grafts in view of the progressive degeneration and disappearance of this tissue following long periods of implantation (Deterling Creech, and others) The over-exposure of aortic grafts to ethylene oxide produced significant damage to the elastic tissue and after implantation aneurysm formation was frequent (Sewell and others) Use in the aorta of auto- or homologous tissue other than aorta in which little or no elastic tissue was present normally *e.g* vein pericardium, fascia, skin, revealed a high incidence of aneurysms. On the other hand, experimental work by Hiertonn (1952) (187) included tests of tensile strength of aorta before and after storage in nutrient solution and after implantation. He found the implanted graft with surrounding fibrous tissue stronger than the intact host

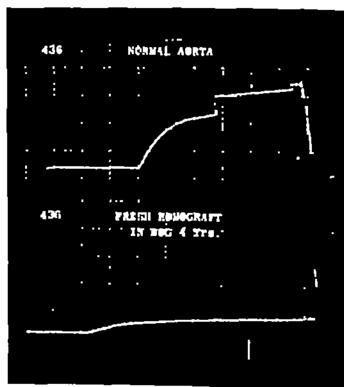



FIG 160 Tension curves of fresh control aorta and of excised homograft which had functioned for 4 years The low flat curve is characteristic of all types of homograft and represents the response to stretch by fibrous tissue and degenerated elastic fibers (Courtesy of C C Coleman Jr and associates Surgery 37: 04 1955 )

vessel. From his data he concluded that most of this tensile strength was inherent in the scar tissue although "it is probable that the often well-preserved elastic structure also makes an important contribution to the tensile strength." He stated further that "the risk of aneurysmatic dilatation following homologous arterial transplantation appears to be insignificant." In a few tension curves of aortic homografts implanted for long periods of time by Coleman and his coworkers (1955) (170) there was a marked difference from those of normal aorta (fig. 160). Less elasticity was noted, and it was the opinion of the authors that grafts implanted for several years in dogs had less tensile strength as compared with the control normal canine aorta. The longest period of observation by Hertoun was 15 months at which time there were a maximal fibroblastic investment and only slight degenerative changes in homografts. Recently Rosenberg and associates (1955) (331) (1958) (331A) have reported good functional results up to two years with bovine carotid heterografts denatured by enzymatic action of ficin before implantation into the aorta of dogs. Of primary interest is the fact that the grafts are essentially dead tubes of a collagen mesh with all elastic tissue removed (fig. 161). Although aortography has revealed a functioning lumen of normal caliber in most of the dogs, one aneurysm has developed. Long term study of these grafts may yield vital information as to the intrinsic value of elastic tissue. In recent studies of heterologous grafts Wesolowski and Sauvage (1957) (332) demonstrated a correlation between loss of elastic tissue and incidence of aneurysm.

*Clinical Use*

An unsuccessful delayed implantation of an arterial homograft into the iliofemoral artery of a patient for luetic aneurysm was performed in June 1910 by Pirovano (106). He mentioned similar cases of Delbet and Doyen but gave no detail. Pirovano used a 10-cm. arterial graft which had been stored briefly in lukewarm physiological serum after removal from a fresh cadaver. The graft thrombosed and the patient died on the sixth day of peritonitis.

*Vessel banks.* Because of problems posed by contamination and infection as well as other factors little interest in preserved grafts was shown clinically until the establishment of an arterial bank by Gross and his associates and their successful use of a preserved homograft on May 24, 1948 (100). This success in an initial series of 15 cases aroused great interest on the part of cardiovascular surgeons throughout the world. Shortly thereafter banks were developed on much the same model throughout North America, in Argentina and in Europe. A report by Keefer and associates (107) described the essential features of a community vessel bank such as the one which was established in New York City in 1950 (fig. 165). Hurwitt (1950) (333) and Cooke and his associates (1951) (334) discussed the establishment of banks for military use. Hyatt and coworkers (1952) (257) outlined various aspects of a tissue bank such as the one which was functioning at the National Naval Medical Center in Bethesda. Subsequently many papers have appeared describing the methods employed in setting up vessel banks in hospitals and communities (Lazzarini, Mortenson and others (335), Henrotin, Creech, Szilagyi, Collin, Kahle (336), Fischer (337), Taber, Perlman (338), Williamson (339), Horton (340), Scott, Bernhard, Flewett, Hipp, Gagnon, Piacentini, Vasquez (341), Benevides and others (342), Castaneda Uribe (343), Rykowski (344), Rams (344A), Baker (344B), Hershey (344C) and others. Of interest is the approach of Provenzall (345) who obtained segments of pulmonary artery from pneumonectomy specimens for his artery bank. In the past few years the preferred storage methods have been freeze-drying or quick-freezing following sterilization of the arteries by irradiation, ethylene oxide or beta-propiolactone. In a few institutions there has been clinical interest in arteries preserved in ethyl alcohol or in formalin.

*Indications for use.* Aneurysm of the aorta and of the peripheral arteries has been a common indication for vessel replacement. Experience with wiring, banding, and wrapping of aneurysms clearly indicated a need for more effective treatment. In September 1951 Dubost (316, 317) successfully resected an arteriosclerotic aneurysm of the abdominal aorta and inserted a preserved aortic homograft. Lacking a bifurcation Dubost attached one iliac artery in end-to-

In 1945 a vein bank was proposed by Blakemore and Lord





Fig 161 *Above* Gross photograph of ficin treated bovine carotid artery showing coarse collagen mesh comprising the outer layers of the vessel The inner surface is smooth by contrast (Courtesy of N Rosenberg and associates A M A Arch Surg 74:80 1957)

*Below* Photomicrograph of ficin treated bovine carotid artery revealing complete removal of the elastic fibers by the enzyme (Verhoeff stain) ×80

rysm of the thoracic aorta, but the operation is more hazardous than that for coarctation or aneurysm of the abdominal aorta. Neurologic complications may be avoided by special techniques. In dogs an intraluminal tube inside the graft as a temporary shunt during implantation was employed successfully by Hufnagel DeCamp, Lam and Aram (371) and others. Lucite polythene, and stainless steel performed equally well. However in fixing the tube in position the aorta may be seriously damaged, and the technique has not become popular clinically Schafer (348) was the first to employ an end-to-side external shunt of polythene tubing in order to bypass the area of resection and replacement of an aneurysm of the abdominal aorta. Alley and others (372) Stranahan and associates (373) Chamberlain (374 375) Adams (376) Sarot and Lazzarini (377) Baffes (378) Flick (379) DeBakey Deterling, and others have used external shunts successfully in resection of aneurysms of the thoracic aorta. In resection and replacement of the aortic arch Stranahan and Alley have used bovine brachiocephalic artery as a temporary shunt, whereas DeBakey and his colleagues have used Ivalon. Unfortunately in these cases and in one reported earlier by Lam the outcome was not successful

Because of the discouraging clinical experience with resection of the aortic arch several experimental techniques have been developed to reduce the risks [Satinsky and associates (380) DiMatteo (381) and Manfredi (382)]. Etheredge and his colleagues (1955) (383) first successfully resected an aneurysm involving the coeliac axis and superior mesenteric artery utilizing an external shunt of polythene. DeBakey and associates (349–352) have successfully resected aneurysms involving the coeliac, superior mesenteric and renal arteries, while using a temporary Ivalon by pass. Hurwitt (383A) had a good result in an aneurysm involving the coeliac axis and superior mesenteric arteries, using a temporary by-pass of ficin treated bovine carotid artery The author had success in employing the same technique in a patient with an aneurysm involving the renal arteries as well In a patient of Sarot and one of the author a by-pass of human homologous aorta was left in place as the permanent channel after resection of an aneurysm of the descending aorta In another patient of the author an aneurysm involving almost the entire descending thoracic aorta was resected and a nylon fabric graft inserted while a perfectly functioning by pass of nylon fabric 2 cm in diameter, was in place. Nevertheless the patient developed a paraplegia, presumably as a result of a compromise of the main arterial supply to the anterior spinal artery

Another method employed to safeguard the central nervous system during resection of thoracic aneurysms has been hypothermia with and without hypotensive drugs. The use of this technique in the resection of aortic aneurysm has been utilized by Julian (148) Searles and coworkers (384) DeBakey and associates (385), Vaja and associates (386) Gwathmey and Pierpont (387) and others Because of specific dangers of hypothermia, the method has not become popular and has been abandoned by DeBakey s group (387A 387B) in favor of partial or complete extracorporeal circulation with the DeWall Read pump-oxygenator apparatus Gerbode *et al* (387C) reported success with this technique in four cases of traumatic aneurysm of the thoracic aorta. Despite potential risks associated with the use of the method the serious nature of aneurysms of the thoracic aorta would seem to warrant further trial of this technique

Occlusive peripheral vascular disease has proved to be more difficult to treat surgically than aneurysms, because of the greater incidence of failure from thrombosis. Although Leriche suggested the use of grafts for the treatment of terminal aortic thrombosis, it was not until 1951 that Oudot (388) achieved this feat. Unfortunately one iliac of the bifurcated aortic homograft thrombosed but Oudot and Mathivat (389) subsequently reported completely successful cases. Excision of the occluded segment and insertion of a graft have been accomplished in the aorta as well as in peripheral arteries, often with partial thromboendarterectomy at the site of the anastomoses, by Hufnagel, Shaw and Wheelock (151) Julian, Dye and associates, DeBakey Creech and associates (385) Rob and Eastcott (390) McAllister and Deterling Hoye and Warren (156) Brintnall and associates (391 392) Wilson and associates (393) as well as by Bahnson Horton, Armstrong (394) Hardin (395) Littman and Soltesz (396) Garbon and Kocnoglu (397) and others. Of interest have been patients claiming improvement of urinary incontinence or libido following restoration of blood flow in the hypogastric arteries (Tocker and Cauble (398) Deterling and others)

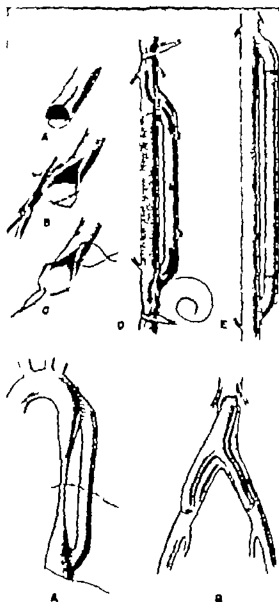



FIG 104 *Above* Technique of preparing ends of arterial segment prior to end to-side anastomosis as a bypass homograft for segmental occlusion of a peripheral artery (A–D) With long occlusions the length of the graft may be constructed of synthetic fabric tubing with homograft ends (E) *Below* Success has been achieved with bypass aortic homografts for a long coarctation (A) or at the bifurcation for arteriosclerotic occlusion (B) (Courtesy of R A Deterling Jr AMA Arch Surg 76 21 1958)

Impressed by the bypass technique employed with venous grafts by Kunlin and associates (1951) (128) Cockett (399) tried this procedure and was also successful with arterial homografts With the use of skip grafts most of the collateral arteries are retained and the surgeon has a relatively undamaged portion of the arterial wall available for anastomosis Like Cockett, Linton (159) compared functional results of end to-end and end-to-side grafts and found that the bypass type was much more successful The report by Hoye and Warren did not mention this difference Crawford (162) Laufman (400) Deterling (163) and their respective associates have obtained excellent results with this method in segmental occlusion of peripheral arteries and the technique has also been successful in the abdominal aorta (fig 164) Mahorner and Spencer (1954) (401) reported on experiments with bypass grafts in the thoracic aorta of 22 dogs. They also reported a clinical case with aneurysm of the innominate artery The use of arterial homografts in unusual location deserves mention Southwick (1950 1951) (402 403) compared fresh autografts of vein with fresh and preserved arterial homograft which were used as portacaval shunts in dogs The arterial grafts showed a higher incidence of failure due in large degree to constriction at the anastomoses Reveno (1955) (404) inserted a preserved arterial homograft into the involved innominate vein during a radical mastectomy operation and noted functional success during 14 months of observation. Denman (1955) (405) reported successful implantation of freeze-dried grafts in a patient with bilateral thrombosis of the carotid arteries Poutasse and his associates (1956) (406) described the first bilateral implantation of homografts into the renal arteries for partial occlusion associated with hypertension The findings in patients re-operated because of a thrombosed

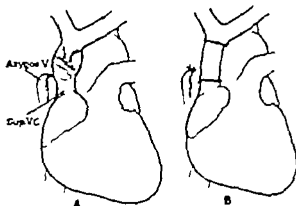



FIG 105 *Left* Drawing to show area of complete obstruction of superior vena cava by tuberculous nodes *Right* Appearance of vena cava following resection of occluded segment and insertion of preserved aortic homograft See cavagram in next illustration



FIG 165 (Continued) Cavagram showing site of obstruction and extensive venous collateral circulation. *Below* Cavagram 6 months after insertion of aortic homograft Despite slight narrowing of the graft there is no evidence of obstruction to blood flow (Courtesy of R. A. Deterling Jr and S B Bhonslay Surgery 38 1008, 1955)

graft have been described by Eastcott and by Hradec

Deterling and Bhonslay (1955) reported on a patient with obstruction of the superior vena cava arising from old tuberculous nodes. The involved segment was resected and an aortic homograft was inserted with complete relief of signs and symptoms. The graft was functioning well at three years (fig. 165)

The necessity for repair of congenital anomalies of the great vessels stimulated the first successful clinical use of preserved arterial and aortic homografts. In Gross first report (1949) (203) 15 cases were described Nine patients had tetralogy of Fallot and a graft was placed between the pulmonary artery and aorta so as to produce a shunt. In the other 6 patients grafts were used to bridge long aortic defects following

excision of a coarctation. Cross' youngest patient with an aortic graft was 7 years old (1951) (399). Lazzarini reported on a 9 year old patient. Occasionally a long stenosis occurs just above the diaphragm and requires a graft for correction (Beattie and associates 1951. Deterling, 1953). More uncommon is a stenosis involving the infradiaphragmatic portion of the aorta [Glenn and coworkers 1952 (407)]. Most well equipped hospitals having access to graft material show that about 10 per cent of the patients with coarctations resected need graft replacement. Clinical reports of coarctation treated by homograft replacement have been frequent. Of special interest has been the use of formalin fixed grafts by Nuboer. The use of arterial homografts has been uncommon in the treatment of tetralogy, and few reports have been encountered (Gross; Palma Rodrigues (408)). Investigators have employed preserved homografts from right ventricle to pulmonary artery in order to by pass the pulmonary valves (Peirce; Hufnagel). Homografts have also been used between left atrial appendage and left ventricle [Scalla and Gulli 1954 (409)]. In general these shunt grafts have thrombosed in time. Manfredi (1954) (410) and Baffes (1956) (411) described techniques which employ arterial homografts for correction of transposition of the great vessels.

Blum, Medl, and Keefer (1956) (412) described the case of a woman who had ulceration of the abdominal wall resulting from congenital absence of the inferior vena cava below the renal veins. A bifurcation homograft was inserted between the upper and lower veins with a good result. However some months later recurrent ulceration was observed and studies were planned to determine whether the graft had thrombosed.

Although few reports of them are found in the literature, traumatic lesions of the arteries have often been repaired by homograft replacement, especially in the years following the Korean War. Although vessel replacement by homograft was not employed by military surgeons during World War II, a relatively small number of patients were so treated for arterial injuries during the Korean War. Some Army cases were described by Cooke, Hughes, Jahnke and Seeley (141). Lahutte and associates (1956) (413) reported on the use of preserved arterial homograft in 21 patients during military campaigns in Indo-China. Additional reports have come from Latin America (414, 415). A torn abdominal aorta required graft replacement in a patient reported by Van Weel (286). Traumatic aneurysm have not been common and often have not required vessel grafts. Oudot and his coworkers used a bifurcation graft preserved in nutrient solution while repairing an innominate aneurysm under hypothermia. Nevertheless the patient died from damage to the central nervous system. Hipp (206) described a patient with a large abdominal aneurysm resulting from a mule kick wherein an inlay aortic graft was used. The sac abutted on the renal arteries preventing placement of the clamp across the aorta below them and failing to provide a cuff for the usual anastomosis.

Involvement of major arteries or the aorta by neoplasm also has required resection and vessel replacement. Swan and Morfit (1951) (207) reported successful resection of malignant tumors involving peripheral arteries and successful use of preserved homografts, restitution of blood flow being achieved. The follow-up period was one and one and a half years respectively. Linton also had experience with graft replacement after excision of neoplasms. Deterling had a patient with a large retroperitoneal tumor involving the lower abdominal aorta and left ureter. In March 1953 a complete resection including left kidney and spleen was performed and a bifurcation homograft was inserted. Stout believed the neoplasm was an adenocarcinoma developing in an embryonal rest. The patient died two years later following an operation on the nervous system. At autopsy, although surrounded by recurrent tumor, the graft was patent and appeared to be in excellent condition, grossly and microscopically (fig. 166). Shortly, S. W. Moore resected a recurrent carcinoma of the colon which involved the aorta and inserted a bifurcation homograft. The result was satisfactory and the patient was alive and well five years following operation.

Aortic homografts have been used in the superior vena cava for obstruction resulting from tumor. Holman and Steinberg (1951) (416) resected a segment of cava for obstruction following radiotherapy for a tumor. However at operation no malignancy was found. An aortic homograft was inserted and signs of venous occlusion disappeared postoperatively. A rise in venous pressures in the arm about a year after operation suggested that the graft had thrombosed, but the patient had no other symptom. Hanlon [cited in (109)] reported

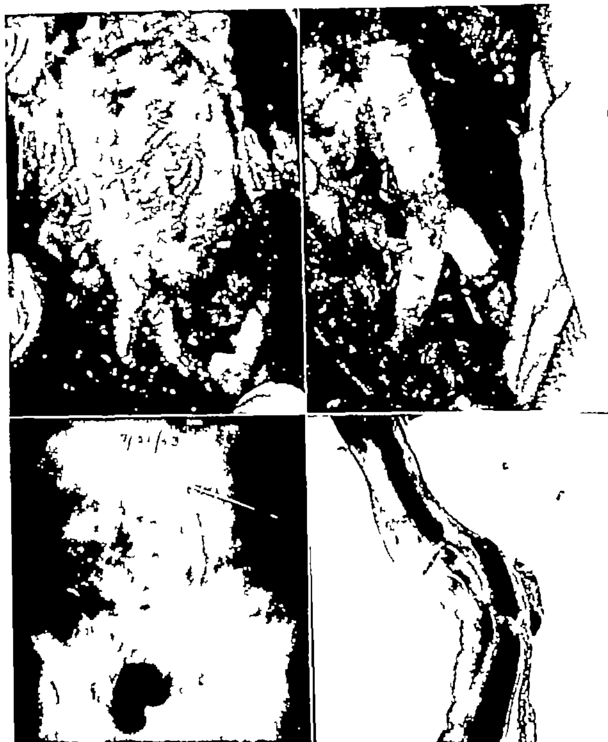


FIG 106 *Upper left* Large spheroid tumor investing the terminal aorta is shown with the common iliac arteries extending below the inferior margin of the neoplasm. *Upper right* Aortic bifurcation homograft has been inserted following removal of tumor together with bifurcation of aorta, spleen left kidney and ureter *Lower left* Aortogram obtained 16 months postoperatively There has been little change in appearance since time of implantation *Lower right.* Photomicrograph of normal aorta and homograft 2 years after implantation There has been firm fibrous healing and organization of mural thrombus in the region of the anastomosis The condensed elastic framework of the graft is seen at top (Verhoeff stain ) ×20

successful use of an aortic homograft in the superior vena cava after resection of an obstructing malignant tumor. The graft was patent when the patient died of the disease approximately eight months later. Higginson (1950) (417) employed aortic homografts in three patients with obstructing tumors. In one resection and insertion of the graft were performed, whereas in the other two by-pass of the tumor was achieved with aortic homografts. DeBakey has mentioned a patient with sarcoma involving the abdominal aorta and inferior vena cava in whom two bifurcation aortic homografts were implanted after resection of the tumor and involved vessels.

*Pacheco et al. (1957) (417A) reported the use* of preserved aortic homografts in two patients with malignant obstruction of the superior vena cava. Blakemore *et al.* (1956) (417B) mentioned the use of an aortic homograft in the superior vena cava with a poor result. Barnes *et al.* (1958) (307A) also mentioned one such case but did not report on the result.

*Complications in aortic homografts.* As has been amply indicated in most experimental and clinical reports of arterial homografts, the most frequent complication has been failure from thrombosis. At times this has been the result of imperfect technique of implantation or of infection at the site of the graft. More often rupture and hemorrhage have been the sequelae of these particular problems. Less commonly thrombosis has developed at the site of the occluding clamps, as was described in the very first experiments by Jaboulay and Briau in 1896 (1). In clinical practice thrombosis of homografts has most frequently been associated with use in patients having peripheral arteriosclerotic occlusive disease, in which instances difficulty in performing a satisfactory anastomosis and the character and distribution of the patient's disease have been contributing factors. Rupture of the graft and aneurysm formation of the graft have been reported infrequently (Brock (370), Hamblin and Lord (330), Cwathmey and Thompson (418), Szilagyi (315A), Barnes *et al.* (307A) and *others*). Some of these failures have been associated with dehiscence of suture lines or with localized damage to the graft related to actual technique of preparation and storage. Fracture of the wall by too rapid and prolonged freezing has been mentioned (fig. 156). However, in 1957 a survey by the Society for Vascular Surgery indicated that success had been achieved with homografts in the aorta in more than 90 per cent of the cases, a figure which speaks favorably for this type of vessel replacement (418A).

## Homografts of Vein

### *Experimental Studies*

In 1912 Carrel reported on an experiment in which a segment of jugular vein after refrigeration for twenty-four hours was transplanted into the thoracic aorta of another dog. When examined after functioning for 80? days the graft presented an endothelized inner surface and fibrosis of the wall. No smooth muscle *nor elastic tissue remained. In 1915 Blakemore* and Lord (67, 68) reported studies with homologous femoral vein grafts in dogs. The vessels had been quickly frozen and preserved in dry ice up to three months before implantation into a femoral artery by the non-suture technique employing flanged tubes of vitallium. The grafts remained patent for more than a month. The authors considered this period to be sufficient for adequate collateral circulation to develop in a clinical case and hoped to obviate need for amputation. This technique aimed at use in the war zone, never was employed clinically with homografts. In 1910 Donovan (69) by a non-suture technique implanted homologous veins preserved briefly in nutrient solution from the left pulmonary artery into the right ventricle. All grafts were occluded when examined more than a month after insertion.

In 1951 Macpherson and associates reported studies of homologous grafts of inferior vena cava implanted by suture technique into the aorta of dogs during 1948 and 1949. The vessels had been stored in Simms' X7 solution with 10 per cent homologous serum and the animals were observed up to five months following implantation. The grafts developed moderate mural thrombi but many remained patent. The microscopic pattern of response was similar to that observed in aortic homografts. Following the early inflammatory response active fibroplasia produced a new intimal zone and loose adventitia. Within a day or so of implantation there was loss of the endothelium of the graft and a gradual loss of smooth muscle cells. The elastic fibers were present during the period of observation although they became fragmented (fig. 167). The functional result and fate of these

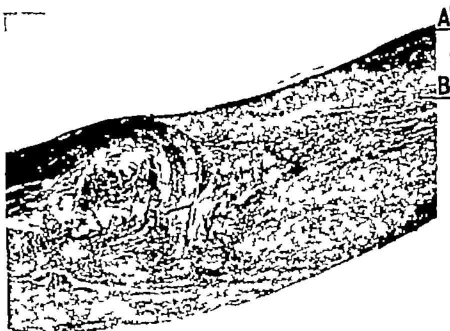



FIG 167 Photomicrograph showing anastomosis of canine aorta (left) to caval homograft (right) at 4 weeks A thick now fibrocellular intima covers the graft (AB) (Hematoxylin and eosin stains) × 10 (Courtesy of A I S Macpherson and associates Arch Surg 63 152 1951)

grafts could not be correlated with the findings of tissue culture of the graft at the time of implantation.

Also in 1951 Oudot described changes observed in fresh and preserved venous homografts in the aorta of dogs. In frozen grafts examined after six weeks histologic differences from fresh homografts were slight. There was a fibrin and cellular intimal zone, with thickening of the graft resulting from fibroplasia in the adventitia. Sauvage Harkins, and coworkers (1952 1953) (20 22, 23) compared fresh and preserved (nutrient solution) venous and aortic transplants in the aorta of growing pigs. No failures were encountered in 34 preserved venous homografts nor was dilation observed On the contrary both fresh and preserved venous homografts decreased in caliber or remained unchanged Histologically both types became acellular and heavily invested by fibroblasts from the host.

Salem (1953) (410) studied caval and iliac venous homografts implanted into the aorta of dogs after storage in a nutrient solution. In some animals a specially constructed thin polythene tube lined by vein was fixed in the aorta by ligatures Southgate, Fomon and Mahoney (1955) (96) studied the behavior of venous auto- and homografts in the thoracic aorta of dogs. Segments of inferior vena cava, 1 5 to 5.3 cm. in length, were preserved in a nutrient solution at 4°C for 1 to 56 days before use. Two of three homografts without external splinting dilated in the aorta and after one year they had developed calcific and atheromatous plaques. In 8 dogs observed from 6 to 270 days after implantation of preserved cava splinted by tantalum gauze, 4 completely thrombosed one of these and one other ruptured In only two without thrombus there was mild dilation in one and aneurysm in the other In 7 dogs, observed from 3 to 56 days after implantation of preserved cava splinted by Ivalon, 2 to 4 mm. thick, none completely thrombosed and none ruptured. However moderate dilation occurred in two and mild dilation developed in two others. The microscopic changes were those described by the previous investigators.

In 1958 Bryant *et al.* (419A) studied replacement of the inferior vena cava in dogs and had very poor functional results with fresh or freeze dried venous homografts, in contrast to those with fresh venous autografts.

*Clinical Use*

In 1953 Dye and coworkers (136) reported on the use of venous grafts in 30 patients with peripheral occlusive arterial disease and their follow-up for periods of 17 to 23 months Among 16 successful grafts there were 4 homologous saphenous vein transplants. Among the 15 failures 3 were venous homografts. In a subsequent report 4 late closures were added of which

2 were of homologous venous grafts. Julian and his associates (1955) (148) used one venous homograft in a series of 9 patients treated for *popliteal aneurysm. In 1955 Shaw and Wheelock* (151) reviewed their experience with grafts in the treatment of occlusive disease of the femoral artery. Among 13 venous grafts employed, one was a frozen homograft. Eight grafts failed but it was not stated whether the homograft was among them. In most clinical series the preference has been for fresh autogenous vein or preserved homologous artery rather than for preserved homologous veins, which lack the best qualities of the others and which most often have been segments of abnormal (varicose) veins.

## HETEROLOGOUS BLOOD VESSELS

### Transplantation of Fresh Artery

#### *Experimental Studies*

In 1903 Höpfner (2) implanted fresh grafts of aorta from one rabbit and two cats into the femoral artery of dogs by non-suture technique but all of the implants thrombosed. In 1907 *Guthrie (45) successfully transplanted two fresh* heterografts. One, a segment of feline aorta, functioned well for 50 days in the carotid artery of a dog. The other, a length of rabbit aorta, implanted into a dog was satisfactory during the 31 days of observation. The next year Ward (107) made a detailed report of a rabbit *aortic graft implanted by the Carrel technique* into the carotid artery of a dog for 70 days. Grossly the graft appeared dilated and slightly thinned. Histologically all normal structures had almost entirely disappeared. The elastic fibers were no longer present. Capelle (1908) (4) claimed functional success with arterial heterografts but noted degeneration at 40 days. In 1908 Stich reported on 12 experiments in which segments *of cat or rabbit artery were implanted* into carotid arteries of dogs. Seven failed from thrombosis or hemorrhage. In 1909 Stich and *Zoeppritz (51) described 4 successful fresh* heterografts. Two were transplants of cat aorta into the carotid artery of dogs with resultant good function for 15 and 51 days respectively. In the first, the intima and inner media were not greatly altered and many fibroblasts invaded the adventitial zone. In the second, the lumen was lined by organized mural thrombus. Apparently fibroblasts arose from the host aorta and surrounding tissues. There was scattered destruction of the elastic fibers of the graft. The third graft was rabbit aorta functioning in the carotid *artery of a dog for 52 days. Mural thrombus and* fibrocellular tissue containing elastic fibers constituted the new intima. There was also *complete destruction of the media of the graft.* The adventitia was replaced by fibrous scar. The fourth graft was human posterior tibial artery in the carotid vessel of a dog which was examined at 14 days. In this brief time there had not been much destruction of the inner medial zone. Endothelium from the host appeared to be growing in from the host vessel. There was moderately heavy fibroblastic infiltration of the adventitia and outer media of the graft.

Also in 1909 Borst and Enderlen (5) included a trial of fresh heterografts among their extensive investigations. A graft of cat aorta and one of goat carotid in the carotid artery of dogs failed. Villard and his associates (1910-1911) (8, 10) reported a 35-day observation of rabbit aorta in the canine carotid artery. There was fibrous proliferation of the intima, especially next to the suture lines, and a fibrin lining. The smooth *muscle and the elastic fibers were fragmented* and degenerated in areas of the media. A moderately severe inflammatory reaction with fibroplasia was present in the adventitial zone. Guthrie published supplementary report in 1910 and 1912 (7, 47) and made an interesting observation on a rabbit aortic graft functioning in the *dog carotid artery for 214 days.* The graft no longer contained any smooth muscle or elastic tissue but did demonstrate calcification and scar tissue that had developed an osteoid appearance. In 1911 Yamanouchi (9) described two cases of successful fresh heterografts among the results of his extensive experiment. *In a canine* *iliac graft functioning 12 days in the aorta of a* cat he noted intimal proliferation with new elastic *tissue as well as fibroplasia in the adventitia.* The media had become necrotic. The other graft was of *feline aorta which had been in the carotid* *artery of a dog for 12 days.* Yamanouchi observed the same intimal and adventitial alteration. The graft itself had become acellular and the elastic tissue was fragmented. In 1913 Carrigliani (11) reported thrombosis of 4 fresh grafts of feline abdominal aorta in the carotid artery of dogs.

Since both functional failure and intense host reaction have been common regarding the

erografts than regarding auto- or homografts interest in heterografts has remained at a low level. Of all the studies in recent years on vessel replacement few have been devoted to fresh arterial heterografts. Tune and Owens (1955) (420) implanted 2 fresh rabbit and 1 fresh feline aortic graft into the aortas of puppies for 7 days, 17 days, and 12 months respectively. Host reaction to these was marked and included adherence of the lung and diaphragm. The grafts were patent, but one had several mural thrombi. No aneurysm developed in the one-year specimen. Hardin (1956) (421) reported very poor results with 7 fresh heterografts of aorta in the aorta or brachiocephalic artery of dogs. One graft each from the monkey, cat, and goat thrombosed. Of 3 human grafts, 2 thrombosed and 1 ruptured. A transplant from a pig became necrotic in 20 days. Three additional fresh heterografts were implanted after irradiation with 300 roentgens to reduce antigenicity. One each from monkey and goat thrombosed, but a pig vessel remained patent and the host dog was continued under observation. Sauvage and Wesolowski (1955) (422, 423) implanted fresh canine aorta into the thoracic aorta of 10 growing pigs. Six developed aneurysm, five of which ruptured. No failures from thrombosis were noted during a period of 260 days. The grafts showed little increase in size except at the site of aneurysm. Fragmentation of elastic tissue, loss of cells, and calcification were noted in all. In 11 other pigs the authors implanted composite grafts comprised of a segment of fresh homologous and one of fresh canine heterologous aorta. Aneurysm developed in 8 grafts but only in the heterologous segments. Marked loss of elastic fiber continuity was observed in all heterologous tissue in contrast to minimal or no loss in the homografts. In later studies the authors have reaffirmed their belief that loss of elastic fiber integrity of the fresh porcine heterografts in the canine aortas was conducive to aneurysm formation (1957).

*Clinical Use*

In several hundred references in the world literature the present author found no reference to the use in a patient of a fresh arterial heterograft nor did he encounter any reported usage of fresh venous heterologous grafts in animals or in patients.

**Transplantation of Preserved Arterial Tissue**

*Experimental Studies*

The first successful transplantation of a preserved arterial heterograft was reported by Carrel in 1907 (40, 41). Two of four segments of canine carotid artery implanted into the abdominal aorta of cats remained patent. One which had been stored for 20 days in saline at 4 C. was functioning at 77 days, whereas the other, preserved in Locke's solution for 4 days before use, was found open at autopsy 6 days after operation. The other two grafts had been refrigerated in defibrinated dog blood before implantation. They thrombosed at 10 and 39 days. The next year Carrel announced good functional results with a segment of human popliteal artery obtained from a leg amputated for osteosarcoma and implanted by suture technique into dog aorta for 7 months and 12 days. The graft had been stored in Locke's solution for 24 days at 4°C. In this paper Carrel mentioned that one of the canine carotid heterografts in cat aorta described earlier was doing well at one year. In another article Carrel (1912) revealed that the human popliteal graft had functioned in the dog's aorta for 4 years and 2 months. When examined microscopically the wall appeared to be hyaline connective tissue. No muscle nor elastic tissue remained in the graft.

In 1908 Levin and Larkin (272) performed experiments aimed at determining whether or not non-viability was the reason for thrombotic failure of grafts. They cited the theory of Brucke which suggested that blood remains fluid as it circulates through a vessel lined with unimpaired and living endothelium, and they believed Carrel's successes to be due in major part to the use of living grafts. They implanted several segments of human artery, devitalized by boiling and then hardened by 4 per cent formalin, into the arteries of dogs and cats. All these grafts became occluded by thrombi. In Moure's thesis (1914) (424) a successful implantation of human popliteal artery into the carotid artery, after storage in saline for 24 hours at 4°C, was described. When studied at 53 days the graft was invested in connective tissue. The muscle cells had disappeared, but some elastic fibers remained.

Little or no work with preserved arteri heterografts appeared in the literature for alm two decades. In 1943 Weiss (425) studied generation of peripheral nerves approxi

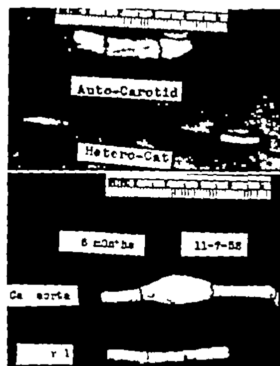



FIG. 168. *Above:* Gross specimens of fresh autogenous carotid arterial control graft (top) and preserved heterologous cat aortic graft in dog carotid artery. The latter (bottom) has thrombosed and nearly disappeared at 7 months. *Below:* Gross unopened specimens of preserved heterologous cat aortic graft in canine carotid artery showing aneurysmal dilation at 6 months. The fresh autogenous carotid arterial transplant from the contralateral vessel is shown at bottom.

inside arterial sleeves in rabbits, cats and monkeys. Better results were achieved in the aorta than in carotid artery because there is less muscle. Weiss considered heterologous artery poor because of the intense inflammatory reaction of the tissues to these segments.

In 1949 Gross, Bill and Peirce (203) described studies of arterial heterografts preserved in Hanks solution with homologous serum and implanted into the abdominal aorta of dogs. One human artery dilated at two months, although it remained patent. Of 4 porcine aortic grafts, 1 thrombosed, 1 developed aneurysm, and 2 were patent at 1 month and at 4½ months respectively. Three aortic heterografts from the baboon thrombosed and actually disintegrated in 4 months. Gauthier-Villars and Oudot (1950) (120) had poor results with calf arteries in 18 dogs, although in 1951 they claimed better results with arterial homografts and heterografts than with autogenous venous segments in the thoracic aorta of dogs (427).

In the same year Colombo, Teich and C...a (328) reported 21 experiments in which grafts of aorta and peripheral arteries were preserved by storage in Hanks solution and serum, by freezing to −25°C and by fixation in formalin. In ... animal, sheep heterografts were observed up to 6 months with only one fair and one good result (at 3 months). Five had developed ... thrombus and one was completely occluded. In 1952 Ohara and Deterling (15) performed unreported experiments in which aortic segments from rabbits, cats, a monkey and a goat were stored in Simms X6 solution with homologous serum at 4°C for less than a month prior to use. After implantation into the carotid artery or abdominal aorta of dogs, most of the grafts failed due to thrombosis or ruptures. One cat aorta which thrombosed was almost completely destroyed. In a few implants there was moderate thrombosis but slight function. One monkey, one goat, one rabbit and two cat vessels retained complete patency (fig. 168). However after 6 months 3 of these showed moderate dilatation (fig. 168). There were severe inflammatory reaction and advanced degenerative changes including calcification and destruction of the elastic fibers in most of the specimen (fig. 16?). In 1952 D'Antona (428) performed implantations of arterial segments from sheep and cow to dog aorta after brief storage in saline. The grafts were inserted with vitallium tubes and although they appeared to behave well, there was a significant number of failures from rupture at the edge of the metallic rings or from the anesthetic agent.

Sautot and his associates (1952) (998) preserved segments of calf carotid artery by several methods prior to use in the abdominal aorta of dogs. The grafts were frozen in Ringer's solution containing Phenergan, chloromycetin, and heparin. Some were stored at −70°C., then the temperature was raised to 4°C at various periods of time. In a series of 9 dogs there were 5 failures. In a later report (1954) the histologic changes in successful grafts were described. These included replacement of endothelium by a fibrinous matrix, fibrohyaline degeneration of the media with disappearance of the muscle cells, hyperplasia of the external limiting elastic membrane and fibrous thickening of the adventitia. Some of these observations were drawn from experiments begun in 1952. These authors mentioned the studies reported in 1952 by Economos and Hewitt (1...

In addition Sautot and his associates cited 2 successful implantations of human iliac arterial segments into the abdominal aorta of dogs after preservation of the grafts for 28 days in Hank's solution at 1°C. One graft was studied at 19 days and the other at 148 days.

Zannini and his coworkers (1953) (430) studied homografts and heterografts implanted into the thoracic aorta of dogs after preservation of the tissues in nutrient solution containing antihistaminic drugs (Fargan, Neohetramina). They reported moderately good functional success with 5 of 6 grafts of lamb aorta, 3 of 4 horse carotid or iliac arterial grafts, and 4 of 4 pig aortic or innominate arterial implants. They discovered however histologic damage in the heterografts. Hufnagel and his colleagues (1953) reported on a series of 21 dogs with heterografts in the abdominal or thoracic aorta. These vessels had been sterilized by exposure to ethylene oxide and then were freeze-dried. Among 10 calf arteries stored 7 to 124 days and observed following implantation for 8 to 402 days, there was only one partly thrombosed. Of 4 pig grafts stored 3 to 162 days before use and then studied for 10 to 177 days, all were patent, but one had a small mural thrombus. One human artery stored for 5 days was patent at 153 days. Among 6 lamb heterografts stored 3 to 82 days and followed after operation for 17 to 170 days, there was 1 failure from hemorrhage and 2 from thrombosis. One additional graft was partly occluded. The authors concluded that satisfactory clinical results could be expected of pig and calf heterografts. Hufnagel later stated that treatment of heterografts by ethylene oxide may reduce their antigenicity (1954) (191).

In 1954 Creech and his associates (287) presented the results of 50 implantations of heterografts from pig, sheep and calf into the aorta of dogs. Forty-four grafts had been fixed in 4 per cent formalin and 12 had been freeze-dried. Of interest was an aneurysm observed at 13 months in a freeze-dried heterograft in the abdominal aorta of a dog. The investigators gave an excellent description of the chronological alterations observed by arteriography and autopsy in 20 formalinized and 8 freeze-dried heterografts over a period of 20 months. All of 13 grafts in the carotid artery thrombosed, as did 3 of 21 in the aorta. Of the 18 patent grafts 9 became dilated. There was gradual deterioration of the heterografts following implantation and an early marked inflammatory reaction. There were extensive areas of hyaline degeneration of the media with calcification by the seventh month. Subsequent osteoid formation was observed in some grafts. "The gradual diminution and final disappearance of elastic fibers, the principal supportive element of the wall of the vessel, result in dilatation." The changes were identical in formalin-fixed and freeze-dried heterografts. "The intense host reaction to all these grafts in some instances amounting to destruction of the graft suggests that after early occlusion the transplants are rejected by the host. The authors concluded that arterial heterografts preserved by the methods used in these experiments eventually weaken and dilate and are therefore unsatisfactory as vascular transplants.

Morton and Mahoney (1954) (431) performed experiments aimed at a reduction in the incidence of aneurysm formation in heterografts. Segments of aorta from young pigs were stored in Ringer's solution at 3°C up to 13 days. After implantation of the grafts into the abdominal aorta of dogs, a sheet of polyvinyl formal sponge was sutured snugly about the anastomosis and the graft. Among 16 animals with grafts patent at least 24 hours, 9 survived 7 weeks to 6 months and were living at the time of the report. Visualization studies demonstrated that no dilation existed, although there were some constriction at the suture lines and irregularity of the lumen. Autopsy on 8 dogs revealed relatively poor results—complete thrombosis in 3, rupture in 2, false aneurysm in 2 and partial occlusion in one. The authors stated that "this percentage of failure is higher than that following use of homografts in similar experiments in this laboratory." Tune and Owens (1954) (420) reported on the implantation of freeze-dried rabbit aorta into the thoracic aorta of dogs. Early transverse fractionation of the elastic fibers occurred with subsequent aneurysm or rupture in animals surviving more than one week. In addition there was marked host reaction to the grafts exhibited by extensive fibrosis and diminution to complete absence of elastic tissue in the media during the period of a year. Kimoto and his coworkers (289) reported on 21 human arterial heterografts in the aorta of dogs following storage in 70 or 100 per cent ethyl alcohol. Two thrombosed completely and mural thrombus was observed in 5 others. The reaction was only moderate and no

aneurysm occurred. The authors considered these encouraging results to be related to a denaturation of lipoproteins of the graft by alcohol, and hence a reduction of antigenicity.

Nicks (1955) (246) made brief mention of the use of two bifurcation heterografts of pig in lamb. The segments had been freeze-dried and were patent when the report was made shortly after implantation. Martino and his associates (1955) (432) claimed good functional results in 20 of 24 dogs with heterografts during a period of a year. The grafts (2 to 7 cm.) from calf, horse and pig were fixed for 10 hours in 4 per cent formalin before storage in saline at room temperature. Distefano and Coscarelli (1955) (433) compared results with homografts and human heterografts in dogs. Six segments of human femoral artery were kept for 5 to 10 days at 5°C. in electrolyte solution containing chick embryo fluid and buffer. After implantation the graft was wrapped with gelatin sponge containing Fargan and this antihistaminic drug was administered for 10 days in an effort to reduce antigenic response. Only one graft failed from rupture or thrombosis but, in contrast to the homografts, all were calcified within a year. The authors believed that a less violent host reaction and good functional results were due to the use of the antihistaminic drug.

In this country Hardin (1955) (421) reported an extensive study of frozen heterografts from monkey, human, cat, goat and pig, with and without preliminary irradiation (300r). Of 6 grafts kept at −70°C. prior to use, 4 completely thrombosed, 1 was partially occluded and 1 (pig) was necrotic at 28 days. In 10 grafts which had been irradiated, 5 (1 from each species) completely thrombosed. Two human heterografts were among the 5 patent grafts. One of these was significantly dilated. Three pig heterografts functioned well for 30 days, 100 days and 48 months respectively. The author concluded that his best results were achieved in pig heterografts, especially those which had been irradiated.

In 1957, Inahara *et al.* (433A) implanted into dogs bovine carotid arteries which had been quickly frozen and irradiated with two million rep." There was violent rejection and failure despite heavy irradiation.

Sauvage and Wesolowski (19..) (123) presented an excellent review of their studies of 230 fresh and preserved homo- and heterograft. Of 12 freeze-dried canine aortic heterografts in growing pigs 3 developed aneurysm, and 2 of these ruptured. In addition 4 became occluded by thrombus. All but one graft showed severe loss of elastic continuity. Of 12 freeze-dried human arterial heterografts in growing pigs, 6 formed aneurysm, and 3 of these ruptured. One other graft thrombosed. All showed marked elastic degeneration. Composite grafts were implanted in 11 pigs. The implants were ... long and each was comprised of a segment of canine aorta preserved in Tyrode's solution and one which had been freeze-dried. In 2 pigs aneurysm developed in each component of the graft, and in 2 others aneurysm was found in 1 part only (1 in the Tyrode part, and 1 in the freeze-dried part). In 10 composite grafts there was serious deterioration of the elastic tissue of both components. In 12 other pigs freeze-dried homologous-heterologous canine composite grafts were studied. Five aneurysms were noted, all of them developing in the heterograft portion. In 10 of the heterografts there was marked loss of fiber continuity in contrast to no loss observed for 212 days in the homografts. This study consistently revealed significantly inferior behavior of heterografts regardless of species, age, or method of preservation. More recent studies (1957) have again correlated aneurysm formation with loss of elastic tissue. Little difference in behavior of porcine and dog heterografts was noted as a result of treatment by ethylene oxide or by freeze-drying.

Szilagyi *et al.* (1956) (433B) implanted bovine carotid arteries into 20 dogs after treatment with BPL and freeze-drying. Six died of hemorrhage and one developed an aneurysm. Thirteen had satisfactory function but there was severe degeneration of the graft. Papp *et al.* (1957) (433C) studied freeze-dried bovine carotid arteries in 40 dogs. Sixteen died of rupture or thrombosis and 12 others had major thrombi or aneurysm in the graft.

*Histologically*, there are certain alterations which can be observed in implantation of arterial heterograft. There is an early inflammatory response regardless of species with appearance of polymorphonuclear cells, lymphocytes and plasma cells (fig. 169). A histologically there is much greater variation in this response than in that observed in any group of homologous aortic grafts studied in the early period. After several days there is generally almost complete necrosis of the heterograft with destruction of a

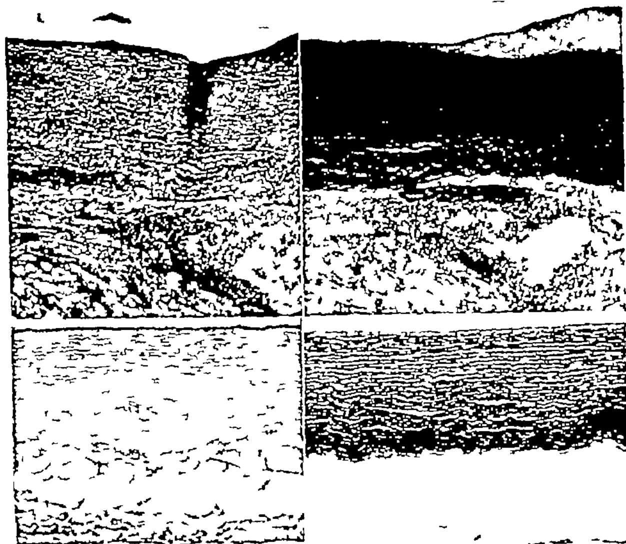


FIG 160 *Above* Photomicrograph of cat aorta in dog carotid artery for 5 days There is an intense inflammatory response in the adventitial zone with many polymorphonuclear cells present Most of the cellular elements of the graft have already disappeared The elastic fibers stain deeply but are still intact × 72 *Below* Photomicrograph of cat aorta in dog carotid artery at 21 days The graft is completely acellular and there is no new intima in the mid portion of the segment There is some fragmentation of the elastic fibers × 84 (Hematoxylin and eosin Verhoeff stains )

cellular elements. In some grafts thrombosis or rupture occurs at this time As fibroblastic replacement ensues the graft may regain strength Continued and often rapid loss of elastic tissue may outstrip fibrous healing, and there may be aneurysm formation

Because of the pronounced host reaction to arterial heterografts, several investigators have studied the effects of controlled enzymatic digestion Rosenberg and his associates (1030) (134) (1038)(331A) subjected bovine equine and porcine aorta to the action of several enzymes including pepsin pancreatin, trypsin collagenase papain elastin and ficin After many critical studies of physical characteristics of fresh and treated tissue they concluded that bovine ficin treated carotid artery was most suitable for long term survival studies in the aorta of dogs (fig. 161) Despite the fact that ficin removes all elastic tissue there was but one aneurysm and one significant dilation during 27 months. Late thrombosis occurred in 2 animals, and thus the rate of successful function was 79 per cent. By contrast, there was only 11 per cent long-term success in untreated bovine carotid heterografts The examination of relatively few specimens revealed much more marked host reaction following implantation of untreated grafts. An acute intense inflammatory response occurred during the first week and was followed by a

foreign-body type of action. This response was well demonstrated at 3 to 4 weeks and was marked by 6 weeks. The treated vessels showed little reaction according to observations of sections obtained at 40 days.

Newton and his colleagues (1956) (435) reported on studies of the enzymatic action of pepsin, papain and trypsin on equine carotid artery. They concluded that pepsin could alter antigenic specificity of soluble proteins. If however incubation at 37°C exceeded three hours the vessel was definitely weakened by pepsin (0.1 per cent) and aneurysm could result.

*Clinical Use*

In 1911 Pirovano (106) mentioned the use of heterograft material by Delbet and Doyen, but no details were given. In 1950 Leger and Oudot (430) reported the case of a young man with a *traumatic aneurysm of the femoral artery.* Lacking homografts the surgeons inserted a *canine iliac arterial heterograft after resecting* the aneurysm. The graft appeared thrombosed when studied by arteriogram one month later. In 1952 Bost, Sautot and Touraine (437) described progress on calf heterografts in dogs previously reported by Sautot and associates. Although 5 of 9 animals died within 5 days with thrombosed grafts the authors considered the results sufficiently good to permit the use of calf heterografts in man. A series of 12 clinical grafts was reported by them in 1954. 11 were used for correction of *segmental obliteration of the femoral or iliac* artery. Among these grafts which had been *stored for about 8 months at −30°C 5 failed* because of thrombosis.

*Hufnagel and his associates (1953) claimed* good results with heterografts in 4 patients. In the first a long thrombosed iliac segment was bypassed with a composite graft of freeze-dried calf and pig artery. Excellent function was recorded at 6 months. Freeze-dried calf heterografts were used for radial and popliteal arterial replacement in two other patients. In the fourth a large iliac aneurysm was replaced by a segment of calf aorta. The authors subsequently employed similar heterografts in several other patients *but a long term follow-up report is not available.*

In 1954 Nyhus and Harkin (438) stated that *Maeta had made clinical use of heterograft* from sheep or dog preserved in alcohol. Two grafts were *said to have been satisfactory at one* year. Kimoto and his coworkers (1954) also reported on heterografts *from the dog or cow* used in 5 patients after storage in 70 per cent ethyl alcohol. *A follow-up extending to 15 ...* indicated a continuation of satisfactory function.

*Creech and associates (1954) (287) reported* the use of a freeze-dried pig aorta in the external *iliac artery after resection of a segment involved* by carcinoma. Arteriogram at 5 months revealed *two-fold dilation of the graft* and at autopsy ... months later the dilated heterograft was occluded by recent thrombus. Hardin (1955) (191) reported removal of an aneurysm of the iliofemoral artery and use of a frozen irradiated (30kg) ... aortic heterograft stored 21 days. The function of the graft appeared to be satisfactory at 9 months. Newton and his associates *(1956) (435)* described a patient with intermittent claudication *resulting from occlusion of the femoral* artery. A by-pass of pepsin treated equine carotid artery was used *from iliac to popliteal artery*. Immediate results were excellent but at 1? days there was sudden pain with the appearance of pulsatile swelling over the graft. At 2 months later the aneurysmal heterograft was successfully replaced by a homograft. The authors thought that the failure may have resulted from weakening of the graft by overexposure to pepsin. Of unusual interest is a clinical case reported by Ashburn, Sewell and Huggins (1956) (325). A segment of superior vena cava occluded by *carcinoma was resected and replaced by freeze-dried pig aorta with satisfactory functional results for 7 months.*

**Transplantation of Preserved Vein**

In 1907 Carrel (40) described the transplantation *of a segment of canine jugular vein into the* abdominal aorta of a cat after storage in saline at 0 to 4°C for a week. The graft *thrombosed* shortly thereafter. In 1915 Copley and Stefko (439) reported the use *of 37 fresh grafts of jugular* vein of chickens and turkeys in the femoral and carotid arteries of dogs implanted by means of a non-suture technique. Nine were patent 2 to 1... days later. Twenty-four were partially or completely occluded by thrombus and 1 had completely disappeared. In 1915 Blakemore and Lord (67, 68) inserted by non-suture technique a human saphenous vein graft into the ... aorta of a dog. It was functioning at 10 days. The graft has been kept below −10°C for 3 weeks before use. Shumacker and ... (??) implanted two human iliac vein segments into the

aorta of dogs after fixation in 10 per cent formalin. They were patent when examined at 121 and 133 days but one was dilated. Among the unreported experiments of Oharn and Detorling (1952) was the use of a segment of human saphenous vein, stored in Simms' X6 solution and homologous serum for two weeks, in the carotid artery of a dog. The graft not only thrombosed but later almost entirely disappeared (fig. 170)

Morton and Mahoney (1954) (431) included in their experiments on heterografts supported by Ivalon a series of 19 dogs with aortic replacements obtained from the inferior vena cava of young pigs. The grafts had been stored in Ringer's solution at 3°C for 13 days. Postmortem examination of 9 dogs revealed that the porcine heterograft was patent in 5 but dilated in several and that one had ruptured and 4 grafts had thrombosed. In 10 surviving dogs, the graft was patent in 8 and all were dilated although there was some constriction at the anastomoses. In one additional animal a diseased human saphenous vein had been used, and this graft dilated to aneurysmal proportion in 4 months, with thinning of the encasing Ivalon sponge. Although the total experience with preserved heterologous venous grafts has been relatively slight, the results are not encouraging No clinical reports have been encountered in a survey of the world literature.

## IMMUNOLOGIC ASPECTS OF BLOOD VESSEL TRANSPLANTS

### Autogenous Vascular Tissue

From the many experimental and clinical observations already described it appears to be well established that fresh autogenous artery or vein tolerates transplantation well and is well accepted in its new location. Functional failures have been exceedingly rare even when arterial grafts, 2 to 3 mm. in diameter have been employed, as free grafts The gross and histologic studies suggest that a minimal inflammatory response of transient nature is followed by normal fibrous repair following the pattern observed in a simple vascular anastomosis. Much of the normal type of nutrition via vasa vasorum becomes re-established as does autonomic innervation. After 3 weeks or more the gross and histologic appearance of such an implant may vary little from the adjacent vessel, provided the transplantation has been artery-to-artery or vein-to-vein

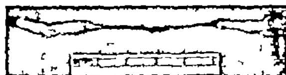


FIG 170 Segment of preserved human saphenous vein in canine carotid artery has thrombosed and almost completely disappeared by 9 weeks

Even when physiologic stress is exerted on the transplant, as occurs when a venous segment is introduced into the arterial system, there may be a response by increase in fibrous tissue and even dilation in time, but no evidence of non-acceptance of the tissue or actual destruction occurs. Autogenous arterial grafts may continue to function and respond to infection as do normal tissues (Bricker)

### Homologous Vascular Tissue

Although there is variation in host response to homologous vascular grafts, the degree of reaction is generally mild. Indeed, Dempster (1951) (440) has stated "blood vessels, I believe are not antigenic If they are antigenic then specific antigens are not strong enough to evoke an immunity reaction of dimensions capable of effecting disintegration. Yet an occasional experimental or clinical homologous graft may evoke a marked response leading to destruction of the graft (Peirce, DeBakey and others) On the other hand there are reports attesting to the persistence in canine or porcine aortic homografts of ordinarily fragile cellular elements over long periods of time Swan and his coworkers (1950) (205 206) described one such homograft in a dog. Parsons, Gerbode and Cox (1952) (16) reported that a canine aortic homograft which had been stored in Hanks solution with serum for 46 days before implantation had a normal gross appearance at 9 months There were smooth muscle cells in the outer media but some fragmentation of elastic fibers had occurred. Sauvage and Harkins (1953) (23) and Kanar and his associates (1954) (318) cited persistence of normal cell structure in a porcine aortic homograft after 321 days. There was a similar response observed in a graft placed in a litter mate These authors believed that the degenerative changes observed in homografts regardless of age or type of storage, are directly related to the nature of the antibody-antigen responses of the host.

Koetgen and Yang (1951) (214) described *persistence of nuclei in the inner and outer portions of the media of a homograft in the thoracic aorta of a dog for one year*.

Deterling and his associates (1952) had previously expressed the view that the pattern of degeneration and destruction was a host response to homografts *per se* rather than the effect of *extrinsic factors such as the presence of viable cells in the transplant or the duration and type of storage*. Longmire and Smith (1951) (441) expressed the view that the acquired immunity theory of destruction of homografts offered a satisfactory explanation of their behavior in various types of tissues or organs.

*Efforts to demonstrate specific antibodies have* not been rewarded. Ingebrigtsen (1912) (169) was impressed by the conflicting reports of early investigators in respect to the gross and microscopic changes in vascular homografts. This author attempted to determine whether isoagglutinins exerted an influence on the final state of the homograft. Using the technique of Epstein and Ottenberg he studied the blood of 40 cats and found interagglutination in 5. Short seg*ments of carotid homografts were implanted in* this group and among another group of 9 without demonstrable interagglutination. The author found no significant differences in the ultimate fate or function of the grafts. Parshley and Deterling (1950) studied the tissue culture response of fresh and preserved human aortic tissue to sera of various groups or *Rh factors*. Although a more luxuriant fibroblastic growth appeared when serum of a type differing from that of the tissue donor was used in the nutrient broth than when matching serum was used, the differences were not critical nor quantitative. Eade was cited by Sylus and Harkins (1954) (438) as *having conducted without success* experiments aimed at demonstrating antibodies in dogs and pigs to vascular homografts.

Joshi and Blumenthal (1958) (441A) found a beneficial effect of dehydration and freezing on the lymphocytic response to subcutaneous implants of the adrenal gland and aorta in rat.

## Heterologous Vascular Tissue

The data show rather consistently that moderate to severe host response may be expected to follow implantation of vascular heterografts. Some investigators studying dogs have observed a greater reaction to heterografts of some species than to those of others. M[illegible] *aorta has been poorly tolerated in dogs* (C[illegible], Hardin, Deterling) a[illegible] have lamb and goat arteries (Cr[illegible], Hufnagel, Hardin, Deterl[illegible]). Cat and rabbit arterial heterografts have [illegible] behaved well in dogs, especially in long-term studies (Höpfner, Carrel, Yamanouchi, Deterling, Tune and others). The most promising functional results have been obtained with grafts of th[illegible] pig, horse and calf aorta. The anatomic and histologic composition of the vessel may in part be responsible for this difference. Neverthele[illegible] the pattern of response is one of destruction and rejection when fresh grafts from these species or those preserved by freezing or in nutrient solution are implanted in dogs.

In an effort to affect the antigenic potential of homografts and heterografts certain investigators have treated the tissue by various methods prior to implantation. Zannini employed antihistaminic drugs in the storage solution but without remarkable result. Barberio and others have studied the effects of cortisone and concluded that it was ineffective. Hardin irradiated frozen heterografts with 300 roentgens before *implantation and believed there were beneficial* results. His series was too small for definitive conclusions however. Inahara found no benefit from heavy electronic irradiation. Hufnagel believed that his satisfactory result with freeze-dried heterografts were due to a reduction in antigenicity by pretreatment with ethylene oxide. *Pate, Sewell, Hallen and others* have considered freeze-drying an effective means of diminishing antigenicity possibly through removal of soluble antigenic protein material during reconstitution or by denaturation of protein. Studies by Wes[illegible]low ki and Sauvage (1957) have failed to show any difference in behavior of porcine hetero*grafts in dogs on the basis of either treatment* by ethylene oxide or by freeze-drying. Kimoto has considered treatment with ethyl alcohol an effective means of reducing antigenicity. Finally Rosenberg and Newton have independently favored enzymatic treatment of heterografts as an effective means of reducing the antigenic response.

Studies by Joubert, Bost and Sautot (19[illegible]) (442) were designed to demonstrate host (dog and man) reaction to calf artery. The authors reported two serologic reactions in dogs, by complement fixation or passive hemagglutination, and one allergic skin test in a patient [illegible]

use of heterografts in the arterial system. Little work has been aimed at preliminary desensitization although unreported exploratory studies have been conducted by Pate and his colleagues.

Lazzarini and Lord have demonstrated positive skin reactions to antigen produced from aortic tissue and injected intradermally into patients with arterial homografts

## SPECIAL TECHNIQUES FOR THE INVESTIGATION AND IMPLANTATION OF BLOOD VESSEL GRAFTS

The general criteria for evaluation of vascular grafts before and after implantation have already been mentioned. Estimates of viability have been achieved through the use of tissue culture, contractility response uptake of isotopes, and measurement of oxygen consumption. Arteriography has given valuable information regarding patency The use of special histologic stains or histochemical techniques has added to an understanding of tissue alterations consequent to storage or implantation. Study of transplants by determination of potential differences and of tensile strength has added to our knowledge. Special equipment has been developed to study elasticity of blood vessel segments (Feitelberg and his associates (443)) The use of injection techniques has clarified the revascularization of transplants. By establishing stress conditions by hypervolemia (Gentsch) or by hypercholesterolemia (Creech) the differential behavior of various types of graft has been studied during an accelerated degeneration.

Suture technique was frequently a major objective of the research program in the early years of this century (Carrel, Yamanouchi Goodman and others) Development of non-suture techniques or of rings to facilitate vessel anastomoses has been a product of work by Höpfner Blakemore, Lord (444) and Stefko Poth and his coworkers (445) Oudot, Albert, and others. Additional methods for insertion of grafts without total occlusion of blood flow have been aimed at replacement of the thoracic aorta These have included temporary intraluminal shunts and external shunts (Hufnagel DeCamp (446) Flick, Schafer Lam Chamberlain, Alley McCune, Mahorner and others) Hypothermia, with or without the use of hypotensive drugs and more recently partial or total perfusion by extracorporeal circulation have been adjuncts which may ultimately permit safe correction of aneurysms of the arch of the aorta (Julian, Vaja, Cooley and others)

With reference to the graft itself the inlay technique has been developed which obviates resection of the host segment involved by aneurysm [Freeman, Blakemore Hipp and Brocca (447)] Fresh autogenous arterial grafts of suitable diameter have been contrived from narrow expendable arterial segments (Hurwitt, Sandblom (448) Lazzarini, Schmitz and Potts) Special techniques have been developed to produce bifurcation grafts from straight ones (Lazzarini Poth and Fisher) As a result of the experimental observation that venous grafts in the aorta dilated as did certain other tissues employed for vessel replacement, studies were undertaken to test certain materials employed for external support of grafts. Fascia pericardium skin, nylon net, Ivalon, and even intestine have been used (Sako Johnson Chun Vargas, Hammer (449), Horton and others)

In the treatment of aortic aneurysm of the descending thoracic aorta permanent by pass with exclusion of the lesion has proven to be efficacious (Adams, Sarot, and Deterling) Previously a similar technique had been employed with peripheral vessels in the treatment of occlusive disease. An interesting approach to restoring blood flow to one extremity with segmental occlusion of the iliac artery has been described by Warren (1956) (450) An arterial homograft has been used between the splenic artery and distal iliofemoral artery The by pass graft of vein or artery conducts blood past the obstructed segment without need for its excision or the sacrifice of collateral circulation (Kunlin Cockett, Linton Crawford Warren, Deterling and others) A similar technique has been designed for transposition of the great vessels or for unusual types of coarctation (Baffes Deterling) and for treatment of obstruction of the superior vena cava (Higginson) The concern that small branches of a graft might leak following implantation led to development of a pressure injector which tests the anastomosis before closing [Jentzler (451)] Hiebert and Linton (1957) (451A) described an instrument for preparing arterial homografts Reduction of the disproportionate diameter of a graft for better conformity with host vessels has been accomplished by plication (Schmitz) As these special methods of study and surgical technique become suitable for clinical application the vascular

and sacrifice after a comparable period of time. Adler (1955) (455) reported a similar study, but he bound the net to the pericardium by a thrombin-fibrinogen coagulum. No remarkable changes were observed in 35 dogs during the first year

### Skin

In 1952 Detering and Bhonslay made studies (unpublished) of full and split thickness autogenous skin tubes implanted as replacements in the thoracic aorta of dogs. With the dermis as the inner surface a few of the grafts remained patent (fig. 173) However, all of the split thickness grafts ruptured and most of the full thickness ones thrombosed. In 1955 Hardin (456) described eleven experiments with tubed dermal grafts in 11 dogs. Early thrombosis did not occur in some of the replacements of thoracic aorta despite an absence of endothelium However 3 of 4 grafts in the abdominal aorta were occluded in 30 days, and all 3 iliofemoral grafts were occluded in 20 days Fisher and his coworkers (1956) (254) studied dermal grafts in the abdominal aorta of 25 dogs for 6 months. In a group of 5 in which the superficial corium formed the luminal surface, only one survived more than 10 days. The others developed necrosis and thrombosis with fatal hemorrhage. Of 20 in which deep dermis formed the lumen 7 retained patency for 6 to 8 months. However aneurysmal dilation was common after several weeks. Eleven dogs died of hemorrhage. Although necrosis of the graft was a frequent observation, less thrombus was present than in the first group of 5 dogs After 2 months the grafts were comprised of acellular collagen with scanty elastic tissue Calcification occurred in 2 cases. Horton and his associates (1956) (457) published extensive studies of autogenous full thickness dermal grafts in the aorta of dogs In 10 animals patches were used with the corium inside the lumen. Four fatal thromboses occurred but the other grafts functioned well up to 205 days. In five dogs similar patches were inserted with the deep dermal surface in the lumen. These all showed excellent function. In 10 other dogs tubular free grafts, implanted in the aorta with the superficial corium inside all thrombosed In another series of 14 dogs having grafts with the deep dermis inward 3 died early of unrelated causes, 3 died of late thrombosis but 8 grafts functioned well. One can conclude from the studies of skin tubes that full thickness grafts with the deep dermis forming the luminal surface yield the best results, but that thrombosis or rupture is too frequent to make their use desirable clinically

In 1957 Kittle and Taquechel (458) described preliminary experiments with tubes of chamois, skiver and pickled lambskin in the thoracic aorta of dogs. Although hemorrhage or empyema followed the use of skiver and lambskin, some encouraging results were noted with chamois. There was remarkably little tissue reaction, and good fibroblastic infiltration of the porous material was observed. In 4 weeks good healing and endothelization had occurred, but subsequently a tendency towards basophilism suggested possible later calcification or cartilage formation Observations were limited to less than 6 months however

Al Naaman (1956) (458A) inserted autogenous grafts of full thickness skin into the aorta of 24 dogs. All failed, with thrombosis occurring in 21 and rupture in 3 The longest survival was six months. Pratt (1958) (153C) reported thrombosis, dilatation or rupture of autogenous skin grafts in the aorta of 16 dogs

### Intestine

Fava and his associates (1956) (459) reported on the use of intestine in the thoracic aorta of dogs. Many details regarding the grafts were omitted since the study was concerned with heparinization. Pratt (1958) (153C) observed hemorrhage or slough of bowel used for aortic replacement in 14 dogs.

## USE OF BLOOD VESSEL SEGMENTS IN SITES OTHER THAN THE VASCULAR SYSTEM

### Ureter

In 1908 Payr (400) mentioned the use of a free autogenous vein for ureteral replacement by Tietze (401) Makkas (cited in 462) and Melchior (463) These experiments were repeated much later by Rosenberg and Dahlen (1952 1953) (464) Whether the vein was used between the ureter and the sigmoid or the bladder or interposed in the ureter there were usually slough and leakage in 48 hours. In the few which functioned for longer periods there was narrowing and obstruction from fibroblastic replacement and even bone formation. In some, transitional cell epithelium lined the adjacent portion of the graft. Hardin (1954) (465) had failure with

surgeon has valuable additions to his armamentarium for his attack on diseases of the heart and blood vessels.

## OTHER TISSUES EMPLOYED AS BLOOD VESSELS

### Peritoneum

By virtue of its endothelial surface and ready supply as autogenous tissue, peritoneum has been tried in a few instances for vessel replacement. Carrel (1907) tried a tube of peritoneum to replace an aortic defect in a cat. In 1909 Jiano (452) described experiments in which he employed patches of autogenous peritoneum to fill elliptical defects in the abdominal aorta of dogs.

### Fascia

The collagenous tough features of fascia were believed to have sufficient strength to function well as an arterial substitute. In 1952 Cousar and Lam (453) reported 30 experiments in dogs in which rectus sheath was employed for arterial



FIG. 171. Graft of fresh autogenous fascia implanted in thoracic aorta for 14 months. Two saccular aneurysms have developed (upper left and bottom mid portion of graft).



FIG. 172. Graft of fresh autogenous pericardium in thoracic aorta for 11 months. There have been some dilation and development of atheromatous plaques.

repair. In 15 dogs an ovoid defect was patched with apparent success. One animal was living and well at 15 months. Tubular grafts of fascia 2 to 3 cm. in length, were implanted into the aorta of the remainder. Six failed from thrombosis or hemorrhage. Of the 9 survivors 1 was satisfactory at 14 months. In 1952 Hui and Deterling (200) implanted rectus sheath tubes 5 to 7 cm. in length into the thoracic aorta of dogs, but multiple aneurysms were found rather consistently after 8 months (fig. 171). In 1953 Fontaine, Kim, and Kieny (228) introduced a polythene tube into the subcutaneous tissues of dogs. In a few months a tough fibrous tube with a smooth lumen had been produced about the plastic. This was subsequently excised and employed successfully as an autogenous vascular graft. Peirce (1953) produced fascial grafts by suturing the anterior rectus sheath of dogs about polythene tubes. Of the tubes enclosed in the rectus sheaths from 40 animals 10 were considered satisfactory for use in the aorta usually after 5 to 8 weeks. One failed from infection and hemorrhage. Moderate thrombus formed in 2 others. Significant dilation of some tubes was shown early by aortography but did not appear to progress during a period of observation of 30 months. Bätzner (1955) (454) produced fascial tubes in the same fashion placing the polythene tube in the anterior rectus sheath. He transplanted the graft into the aorta at 5 weeks but had poor results. Pratt (1958) (153C) described experiments with rectus sheath lined with peritoneum and anticipated promising results in the canine aorta.

### Pericardium

Sako (1951) (73) observed fusiform dilation of tubular grafts of pericardium placed in defects of the thoracic aorta of dogs. He found that this dilation could be diminished by external support of the graft with autogenous fascia or even with skin. In 1953 additional observations were reported (74) to the effect that after an initial dilation the supported grafts showed no further enlargement. Vargas and Deterling (1953) (90) reported the use of 5- to 7-cm. tubes of autogenous pericardium in the thoracic aorta of 9 dogs. In 4 without external support moderate elongation and dilation occurred during an observation period as long as 220 days (fig. 172). In 5 with nylon net for external support no significant change was detected by aortography

and sacrifice after a comparable period of time. Adler (1955) (455) reported a similar study, but he bound the net to the pericardium by a thrombin-fibrinogen coagulum. No remarkable changes were observed in 35 dogs during the first year

### Skin

In 1952 Deterling and Bhonslay made studies (unpublished) of full and split thickness autogenous skin tubes implanted as replacements in the thoracic aorta of dogs. With the dermis as the inner surface a few of the grafts remained patent (fig. 173) However all of the split thickness grafts ruptured, and most of the full thickness ones thrombosed. In 1955 Hardin (456) described eleven experiments with tubed dermal grafts in 11 dogs. Early thrombosis did not occur in some of the replacements of thoracic aorta despite an absence of endothelium. However 3 of 4 grafts in the abdominal aorta were occluded in 30 days, and all 3 iliofemoral grafts were occluded in 20 days. Fisher and his coworkers (1956) (254) studied dermal grafts in the abdominal aorta of 25 dogs for 6 months. In a group of 5 in which the superficial corium formed the luminal surface, only one survived more than 10 days The others developed necrosis and thrombosis with fatal hemorrhage. Of 20 in which deep dermis formed the lumen 7 retained patency for 6 to 8 months. However aneurysmal dilation was common after several weeks. Eleven dogs died of hemorrhage. Although necrosis of the graft was a frequent observation, less thrombus was present than in the first group of 5 dogs. After 2 months the grafts were comprised of acellular collagen with scanty elastic tissue. Calcification occurred in 2 cases Horton and his associates (1956) (457) published extensive studies of autogenous full thickness dermal grafts in the aorta of dogs. In 10 animals patches were used with the corium inside the lumen. Four fatal thromboses occurred but the other grafts functioned well up to 205 days. In five dogs similar patches were inserted with the deep dermal surface in the lumen These all showed excellent function In 10 other dogs tubular free grafts, implanted in the aorta with the superficial corium inside all thrombosed In another series of 14 dogs having grafts with the deep dermis inward 3 died early of unrelated causes 3 died of late thrombosis, but 8 grafts functioned well One can conclude from the studies of skin tubes that full thickness grafts with the deep dermis forming the luminal surface yield the best results, but that thrombosis or rupture is too frequent to make their use desirable clinically

In 1957 Kittle and Taquechel (458) described preliminary experiments with tubes of chamois, skiver, and pickled lambskin in the thoracic aorta of dogs. Although hemorrhage or empyema followed the use of skiver and lambskin some encouraging results were noted with chamois. There was remarkably little tissue reaction, and good fibroblastic infiltration of the porous material was observed. In 4 weeks good healing and endothelization had occurred, but subsequently a tendency towards basophilism suggested possible later calcification or cartilage formation. Observations were limited to less than 6 months however

Al Naaman (1956) (458A) inserted autogenous grafts of full thickness skin into the aorta of 24 dogs All failed with thrombosis occurring in 21 and rupture in 3 The longest survival was six months. Pratt (1958) (153C) reported thrombosis, dilatation or rupture of autogenous skin grafts in the aorta of 16 dogs.

### Intestine

Fava and his associates (1956) (459) reported on the use of intestine in the thoracic aorta of dogs. Many details regarding the grafts were omitted since the study was concerned with heparinization. Pratt (1958) (153C) observed hemorrhage or slough of bowel used for aortic replacement in 14 dogs.

## USE OF BLOOD VESSEL SEGMENTS IN SITES OTHER THAN THE VASCULAR SYSTEM

### Ureter

In 1908 Payr (400) mentioned the use of a free autogenous vein for ureteral replacement by Tietze (461), Makkas (cited in 462) and Melchior (463) These experiments were repeated much later by Rosenberg and Dahlen (1952 1953) (464) Whether the vein was used between the ureter and the sigmoid or the bladder or interposed in the ureter there were usually slough and leakage in 48 hours. In the few which functioned for longer periods there was narrowing and obstruction from fibroblastic replacement and even bone formation. In some transitional cell epithelium lined the adjacent portion of the graft. Hardin (1954) (465) had failure with

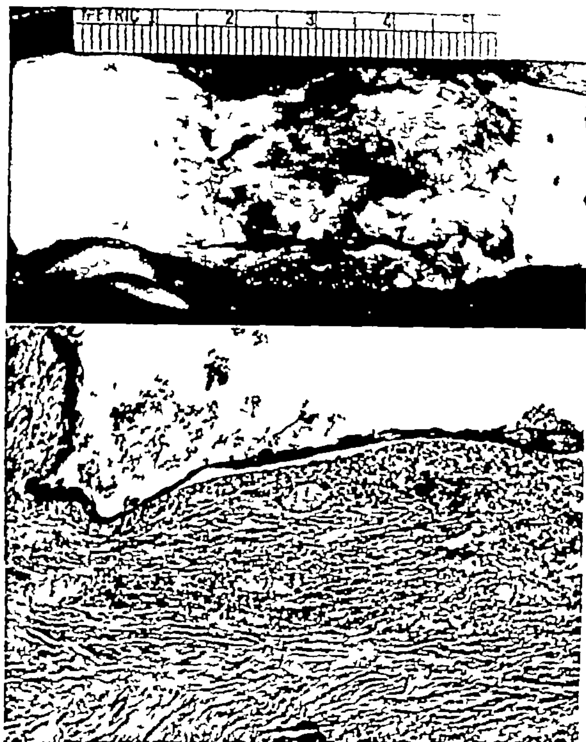



FIG. 173. *Above.* Graft of full thickness fresh autogenous skin in thoracic aorta for 10 months. The deep dermis has formed the luminal surface. The graft has become endothelized following organization of mural thrombus. *Below.* Photomicrograph showing atrophy of epithelium and marked inflammatory changes in skin graft implanted 6 weeks. These alterations are related to infection in some instances. (Hematoxylin and eosin stains.) ×90

free autografts of vein supported by an indwelling tube of polythene. Leakage or stenoses occurred in all dogs, and complete destruction of the graft occurred in one. In 1950 Sanders and Schein (466), who were interested in epithelial morphology, reviewed the behavior of all types of autogenous grafts which had been utilized for ureteral and vesicular substitutes. They observed that tissues possessing a well-developed epithelium-like skin, Fallopian tube, or intestine

will retain the native epithelium despite function of the urinary tract. By contrast, structures with no such lining acquire transitional cell epithelium. Bonome and Begani (1953) and Sewell (1955) (467) tried freeze-dried jugular vein and carotid artery as ureteral replacement but had poor results. In most attempts hydroureter and hydronephrosis ensued along with necrosis of the graft. Sewell observed that this response resulted from irritating chemicals or bacteria and the results could be likened to those seen with vessel replacement of the common duct or esophagus. Calef (1938) evaluated fresh arterial autografts but with no greater success.

*Common Duct*

Much interest on the part of general surgeons centered in the development of a satisfactory type of replacement for the strictured common duct. In 1914 Giordani and Stropeni claimed success with implantation of free grafts of autogenous vein in dogs. In 1943 Lord and Chenoworth studied several types of free autogenous grafts inserted by vitallium tubes and non-suture technique. Fascial tubes for the common duct in 9 dogs functioned during the period of observation although some stricture was seen in 2 cases. No epithelization was noted microscopically. The authors tried femoral vein for duct replacement in 10 dogs, but in 4 there was leakage or absorption of the graft. Epithelization was noted in one examined at 92 days. Four of five peritoneal tubes failed from leakage and disappearance. One was functioning at 2 weeks. In 1948 Shea and Hubay (468) inserted into the common duct free venous grafts in 21 dogs by the same technique. They claimed excellent function in two-thirds of their cases and good results in another 14.3 per cent. Nineteen per cent failed on the basis of obstruction. The authors observed epithelization in grafts examined at 114 and 208 days.

In 1951 Pearce and associates (469) implanted venous grafts by vitallium tubes in dogs but had uniformly poor results. There was marked shrinkage of the grafts from fibrosis. Deposition of bile salts on the inner surface of the cuff and extrusion in some of the metallic prosthesis into the lumen were undesirable features. Hardin and Kittle (1951) (470) implanted grafts of autogenous artery by suture technique over a tube in 9 dogs and without a tube in 6 dogs. All but 1 failed from leakage or stenosis during 14 to 67 days. The authors attempted to place tubes of split thickness skin over a tube in 6 animals, but all failed. Free autogenous venous grafts over polythene tubes were tried by Devin and associates (1952) (471) but only 7 of 20 dogs could be considered suitable for evaluation. Fair results were noted during a 4-month period but the authors drew no definite conclusions as to the value of the method. A clinical case was reported by Olow in 1952 (472). A segment of basilic vein was inserted into a 3- to 4-cm. defect of the common duct. A late biliary fistula healed following demonstration of patency of the graft. However at one year a rise in serum alkaline phosphatase suggested stricture, and at exploration 6 months later it was found that the graft had been destroyed. A hepaticojejunostomy was then performed.

In 1954 Manfredi fashioned common duct replacements from autogenous vein, artery and ureter. Of 4 veins 2 leaked and 2 developed stenosis. Of 4 arteries, 1 was satisfactory at 120 days, but 3 stenosed. Of 4 ureters one was satisfactory at 280 days, but 2 stenosed and 1 leaked. Grugni (1955) (473) employed preserved segments of femoral or iliac artery for common duct reconstruction in 14 dogs. Most of the experiments were failures because of stricture and fibrosis of the graft. Ulm Shoemaker and Entine (1955) (474) described a two-stage technique whereby free venous grafts were threaded over a polythene tube and wrapped in omentum. Six to 12 weeks later the reinforced graft was implanted with a new polythene tube through the spincter of Oddi. There were 2 excellent results at 6 and 9 months. Slight stricture was observed in 1 and partial stenosis was observed in 5. The use of a T-tube seemed to reduce the tendency to stricture.

Santos *et al* (1957) (474A) used preserved aortic homografts to replace the common duct in dogs and obtained fair results with freeze-dried vessels. Surprisingly these functioned better than fresh autogenous artery.

**Fallopian Tube**

Davids and Bellwin (1954) (475) inserted segments of femoral artery or vein into the uterine cavity following anastomosis with the Fallopian tube. In half the animals so treated a polythene tube was left in the graft at the uterine junction. At short-term observations the majority of the

graft were patent and the results were considered encouraging. In 1956 Schein and Ferreira (476) reported studies of 9 dogs in which grafts of splenic or carotid artery were wrapped with Fallopian tube and the end of the vascular tube was left open in the abdominal cavity. Examination between 58 and 113 days revealed all ostia closed and viscera adherent to the free end of the graft. In time there was dissolution of the graft and infiltration by lymphocytes. Ultimately in some animals the graft became a fibrous cord

## Trachea and Bronchus

In 1953 Cadili (477) reported experiments on 6 dogs with arterial patches in the side of the trachea and bronchi. The vessels used were segments of thoracic aorta preserved in 70 per cent glycerine. Cadili claimed satisfactory results.

In 1958 Pressman and Simon (477A) reported observations on the use of aorta in tracheal reconstruction

## Esophagus

Javid (1953) (478) described insertion of fresh and preserved aortic homografts into the esophagus of dogs. In 14 experiments there were 9 survivors with good function in all but 1. At 5 weeks thin epithelium had grown in from the adjacent esophagus and epithelization was complete in three months. Also in 1953 Roux and associates (479) reported 11 experiments in which they employed homografts of aorta or heterografts of vena cava to repair 3- to 8-cm. defects of the esophagus. In 4 cases goat homografts were implanted and in 2 canine homografts were used. Calf and bull vena cava were employed in 5 dogs. Most implants failed because of leakage or stenosis although one calf heterograft was all right at 9 months. The authors believed the degree of stenosis to be proportional to the diameter of the graft used. Epithelium from the esophagus lined the graft of long term survivors. Roux's group envisioned possible clinical application of this type of replacement.

## Intestine

Roux and his coworkers (1953) also inserted segments of goat aorta into defects in the small and large intestine of other goats. The grafts had been stored at 4°C in Hank's solution for 2 to 50 days. In most instances failure resulted from stenosis. It was of interest that the grafts acquired a lining of intestinal epithelium in time

## Tendon Sheath

Koth and Sewell (1955) (480) reported experiments in which freeze-dried arterial homo- and heterografts were used as tendon sheaths in the legs of dogs. The heterografts failed because of intense host reaction and subsequent fibrosis and scarring. Sixteen homografts in 19 dogs were considered completely successful during a period of observation of 3 to 14 months.

In 1950 Gueukdjian (481) reported a case in which a fresh segment of autogenous saphenous vein was used as a tendon sheath for the extensor hallucis longus.

## Nerve Sheath

Weiss (1943) tried fresh autogenous artery as well as homo- and heterografts stored in Ringer's solution or freeze-dried before use in nerve reunion. Heterografts were poor because of marked fibrosis. The freeze-dried homografts appeared to function better than living tissue. While adhesions were observed, there was no undue inflammatory reaction. Weiss found vein segments unsuitable

In 1908 Payr reported experiments aimed at relief of hydrocephalus. It was his hope that free autogenous segments of vein could serve to decompress the ventricles of the brain. Unfortunately lasting function was not achieved

## COMPARATIVE EVALUATION AND CURRENT STATUS OF BLOOD VESSEL TRANSPLANTS

The early investigators handicapped as they were by imperfect technique and threat of infection, nevertheless often achieved remarkable success with vessel grafts as small and fragile as those from the peripheral arteries of dogs or the aorta of cats and rabbits. From most of their studies they concluded that fresh autogenous grafts were superior to fresh homo- or heterograft. When preservation of vessels was feasible the superior result often observed with relatively fresh tissue established the belief that viability of a stored transplant was essential to successful function. This concept was reinforced by the results of the early experiments of recent workers. However additional work with dead frozen aortic grafts indicated that after

factory results could be achieved despite non viability

From studies with quickly frozen grafts a process evolved which has enjoyed successful use in many hospitals. A suitable temperature for freezing has been −74°C with the optimal temperature for storage apparently below −40°C. Although initial vessel banks were established after the model proposed by Gross and associates, employing as it did some balanced electrolyte solution containing serum, the technique made excessive demands on technical personnel and vessel material especially if the storage period was limited to 6 weeks. The introduction of freeze-drying of arteries for hospital banks was particularly important, since this method had special appeal for institutions which supplied material to other hospitals, or for those in which the need might be only occasional. Additional interest in the method arose from observations of some investigators whose data suggested that the freeze-dried artery actually was superior to fresh material when employed as homograft in the vascular system. Unless the processing of the tissue is performed properly there is a real danger of fracturing the graft wall and thus of contributing to subsequent graft failure through rupture. Some such failures have also been attributed to an unusually severe host reaction or to inadequate securing of branches of a graft. Despite the potential danger of rupture or thrombosis of a graft, an accumulated series of patients with preserved aortic homografts has revealed a success rate well in excess of 90 per cent. Infection has very rarely been recorded as the cause of a graft failure despite the fact that most homografts have been secured in an unsterile state and have been sterilized by irradiation or a chemical agent. Sufficient data are not yet available to determine whether homografts fixed in formalin or in ethyl alcohol have been as suitable clinically as those which have been kept in nutrient solution, quickly frozen, or freeze-dried. In the author's opinion, one of the most convenient and satisfactory methods for use in a hospital has been treatment of graft material by betapropiolactone followed by quick freezing and storage at about −65°C.

The status of autogenous venous grafts is not completely settled, since satisfactory results may be achieved with them in peripheral arteries. The percentage of failures with them in cases of occlusive disease certainly indicates that they are not superior to properly prepared arterial homografts. Indeed aneurysm has been the frequent sequel to implantation of autogenous vein in the aorta of animals, and aneurysm has been reported in some few clinical cases in which autogenous venous grafts were used in peripheral arteries. The simplicity in securing them and their availability have probably been more important in their use than their intrinsic excellence. It would appear from the literature that with increasing availability of preserved homografts and the newer synthetic prostheses, use of autogenous vein has gradually diminished. The desire to have bank material upon which to draw at any time and for use in all sites led to a brief reawakened interest in heterografts. Although chemical or enzymatic treatment of heterografts may reduce their antigenicity in general less satisfactory results have been reported with experimental or clinical use. It would be ill-advised to recommend these replacements for human use when better results have been achieved with homografts, autografts and current synthetic materials. The latter hold real promise in the field of vessel replacement, and with the greatly improved synthetic prostheses which became available commercially during 1958, there is an understandable tendency for most vascular surgeons to rely upon them entirely.

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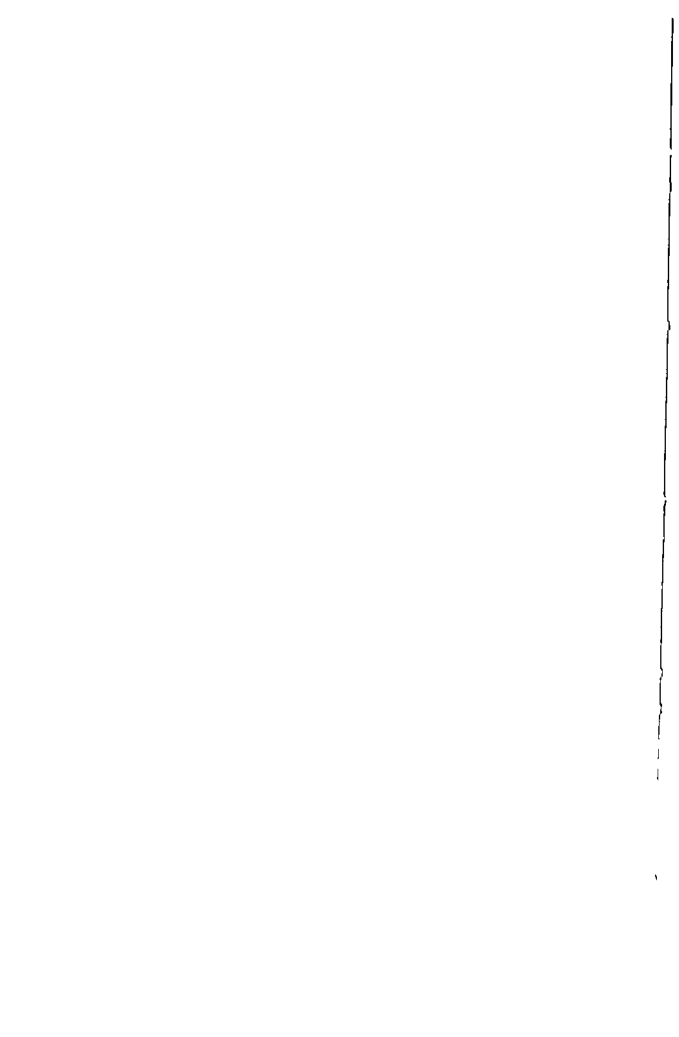
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# PART VIII

# *Endocrine Glands*

CHAPTER 12

# Transplantation of Endocrine Glands

P L. KROHN

## GENERAL INTRODUCTION

John Hunter who played an important part in developing so many experimental methods can rightly be called one of the pioneers in the field of endocrine transplantation, for he had investigated the possibility of transplanting the gonads as early as 1771, although he was naturally unaware of the group of organs we now call endocrine glands. He remarks 'I have also frequently taken out the testis of a cock and replaced it in his belly where it has adhered and been nourished nay I have put the testis of a cock into the belly of a hen with the same effect (1) He also showed that the grafts which adhered to the liver or the intestine were revascularized An eye witness report (2) of some of his experiments says that he had many hens into whose abdomens when young he had put the testis of a cock. Although the grafts increased in size and yielded a white liquor when cut into he was surprised to find that ova were not impregnated, nor was the 'natural disposition of the hen altered. Hunter it is clear did not recognize any differences between homografts and autografts, and seems to have assumed that either type would take readily He also had no idea that the male sexual characters are maintained by a blood borne hormone from the testis

The classical demonstration of the endocrine function of a gland was provided by Berthold in 1849 (3) Berthold first caponized six cockerels which were two to three months old. Two of these were left as controls In another two cockerels, one of the testes was replaced in the body cavity a testis was exchanged between the remaining two All four birds exhibited the normal behavior of uncastrated fowls and their combs and wattles developed normally The grafted testes were seen to be well vascularized and contained spermatozoa.

Berthold concluded from his experiments that the testis could be readily transplanted, even from one animal to another and that either orthotopic or heterotopic transplantation was successful. His other important conclusion was that the testis did not require specific nerves for normal function, and he clearly recognized the fact that whatever substance was produced by the testis must be transmitted to the receptor organs via the bloodstream.

These general conclusions by Berthold represent the first clear formulation of one of the criteria by which we now judge whether a tissue is an endocrine organ or not. It must be possible to remove the effects of extirpating a presumed endocrine tissue by replacing it in the host away from its normal position. The scope of transplantation as a test is however, limited to those tissues which form compact organs that can be dealt with surgically and removed completely It would be impossible to demonstrate by this method the endocrine nature of a diffuse structure throughout say the intestine, which it would be impossible to remove completely or of cells within the lungs whose complete removal would obviously be fatal.

Berthold's important demonstration seems to have failed to stimulate any immediate interest in the use of transplantation and it was not until the end of the nineteenth century that transplantation of endocrine glands first became the popular tool and interest of both experimentalists and clinicians. The general direction of the work has varied from time to time. Thus, at first, efforts

were concentrated on demonstrating that all *endocrine organs could be successfully transplanted* (something which has not yet been fully achieved if one accepts the posterior lobe of the pituitary as a member of the group) and in those days the importance of distinguishing between auto- homo- and heterografts was not recognized or was disregarded There followed a phase in *which transplantation was used mainly as a form* of therapy since it provided the only way to administer to patients in need of them the substances whose secretion by the endocrine glands had recently been demonstrated The very voluminous literature which appeared on this topic particularly in relation to the transplanta *tion of ovaries and testes, is of little consequence* now that the chemists have succeeded in providing practitioners with chemically pure hormones, which are much more suitable for the treatment of most endocrine deficiences nor need the use of testicular grafts to rejuvenate old people detain us.

*More recently transplantation has become a* standard technique to elucidate a wide variety of endocrine mechanisms and interrelationships. Some of these will be discussed later when the endocrine glands are considered individually

It is easy to daydream of a time when the difficult technical problems of the long term storage *of endocrine organs have been solved Even then,* however the replacement of effete endocrine organs would not necessarily become commonplace for any provision of new endocrine organs to replace those which are worn out or defective will be of no therapeutic value until ways and means can be found of avoiding or preventing the *factors which today make successful ex* changes of tissue from one person to another impossible

It is clear that the development of some form of immunity reaction provides an adequate explanation for the events which seem inevitably to follow homotransplantation of such tissues as skin or kidney But endocrine tissues, as a group have often appeared to be less amenable to the forces which deal with, for example a graft of foreign skin Thus a casual glance at the literature concerned with grafting endocrine organs provides an utterly confusing impression in which reports that autografts sometimes fail are mixed with suggestions that homografts often survive and even that heterografts survive To determine whether endocrine organs are uniquely different from the other tissues which have been grafted, and if so *in what ways, represents one of the most import* ant problems which confront the transplanter of endocrine glands.

Closer examination of the literature suggests however that the situation is not as confused as it appears at first sight. The majority of the early workers thought that homografts survived only *occasionally if at all. The idea that homografts* can survive derives largely from later work with purely endocrinologic and not immunologic objectives using laboratory rodents in which the genetic differences between host and donor are often quite uncertain, but where it can usually be assumed that some degree of inbreeding has *occurred. The meaning of the word "homograft"* —and indeed of the word "take —continues to be misinterpreted, and successful grafting of tissues from one member of an inbred strain to another perhaps even within the same litter is still apt to be regarded as proof that homografts may survive. Such homografts are in no way com*parable with the homografts used in skin trans*plantation studies, where every effort is made to insure maximal genetic dissimilarity

Thus the chances of successfully replacing inadequate endocrine organs seem slender at present, though they are not so slim as might have been supposed a few years ago Meanwhile they *justify all attempts to minimize the large* amount of damage and destruction of endocrine cells, which is an inevitable concomitant of transplantation Dempster (310) has often drawn attention to the inadequacies of such "implantation" techniques and has emphasized the advantages of 'transplantation by direct vascular anastomosis *which he employs. It is perhaps* in this direction that the best hopes of the clinical applications of endocrine transplantations lie.

## OVARY

It is usually believed that Knauer (1896) (4) using rabbits was the first person to make successful autografts of the ovary Two years later Ribbert (1898) (5) described how grafts which he had stitched to the peritoneum in guinea pigs developed during a period of up to 135 days after *operation. He was the first to mention the central* necrosis that *follows grafting* and the peripheral proliferation which restores the condition of the graft He did not believe that homografts were successful Herlitzka (1900) (6) was concerned primarily with the problem of the autonomy of

the germ plasm and wanted to find out whether a grafted ovary would be influenced at all by the foreign environment in which it found itself He also failed to transplant homografts in the guinea pig successfully At the same time Foà (1900 1901) (7 8) made the important discovery that the ovary of an immature rabbit which is transplanted to an adult develops very rapidly and becomes functional long before it would ordinarily be expected to do so Conversely an adult ovary transplanted into a young animal will remain inactive until the age of puberty is reached (see p 419 for a further discussion of these experiments in relation to factors which control the onset of puberty and the end of reproductive life) Halban (1901) (9) observed menstrual cycles in baboons with ovarian autografts Menstruation stopped when the grafts were removed. He, like Marshall and Jolly (1907 1908) (10 11) was concerned mainly with demonstrating the endocrine function of the ovary

Others whose interest lay in attempts to achieve pregnancies from grafted ovaries included McCone (1899) (12) Guthrie (1908 1911) (13 14) Castle (1911) (15) Davenport (1911) (16) and Castle and Phillips (1913) (17) Chicks and guinea pigs were used and at various times it was suggested that the offspring which were obtained after ovariectomy and replacement of the host's ovary with an ovarian graft had characteristics which could be attributed only to the host and not to the original donor of the graft tissue. But in all these experiments, and particularly in experiments with chickens it was difficult to be certain that the ovariectomies had been complete nor were the stocks of animals so genetically pure that the coat or feather color changes, which provided *most of the evidence, could be* relied on as satisfactory evidence for any somatic influence on the germ plasm.

Much more recently the technique of intracapsular transplantation of the ovary has been further developed by investigators at Bar Harbor and in their experiments on exchange of ovarian tissue from one mouse to another the appearance of offspring which resembled the host and not the donor has always *been due to regeneration* of host ovarian tissue

Although early workers seem to have been agreed that homografts were unsuccessful (for reviews see Schultz (1910) (18) and Tscherniachoff (1914) (19)) a period of great interest in the use of ovarian grafts of all sorts followed, particularly for the treatment of deficiency conditions in women. Gradually however gynecologists became *less convinced of the value of homotransplants* and interest in the use of such methods has virtually disappeared. How the climate of opinion changed can be seen from a series of reports on the value of transplantation of the ovary provided by Martin (1908 1911, 1915 1917) (20–23) which can be used also as a bibliographic source for most of the work which had been published up to that time. In his first review Martin appears to believe that homotransplantation is of value and that even heterotransplantation may sometimes succeed. By 1917 however he says

> The only form of ovarian transplantation that is practicable is autotransplantation and this has a rather limited field of usefulness in the retardation and modification of the symptoms of the artificial menopause brought about by the complete removal of the ovaries In spite perhaps of the very enthusiastic conclusions of a few workers neither homo- nor heterotransplantation has as yet justified its use in human surgery

In 1925 Bell (24), reporting that symptoms of the menopause disappeared in 98 out of 118 patients with the occurrence of menstruation in 71 out of 107 patients when autoplastic grafts had been used remarks that homoplastic grafting with ovarian tissue from another woman is very rarely effectual, an opinion which had also been put forward earlier by Tuffier (1911) (25) Autografting may still have a limited place in gynecology for the retention of natural ovarian tissue when normal tissue can be identified and recovered in operations which involve removal of pelvic organs (26–30) A complete review of the whole field of ovarian transplantation up to 1928 is given by Pettinari (1928) (31) who also deals with heterotransplantation à la Voronoff and with experimental attempts to rejuvenate the ovaries of old animals by means of transplants.

### Assessment of Function of Graft

The most complete test for a satisfactory ovarian graft is of course the production of offspring by a host whose own ovaries have been completely removed Such a criterion can, however be fulfilled only by an animal which has received an orthotopic graft (or possibly a graft into the uterus itself) Other incomplete tests of reproductive function, such as the occurrence of ovulation in the graft have to depend on histo-

logic examination at the end of the experiment (32). But besides producing ova the ovary is responsible for the secretion of hormones which enable the reproductive tract to bring a pregnancy to term, and a partial test of this function has been developed by Kullander (1950) (33) who examined whether the host was able to maintain the development of experimentally produced deciduomata in the uterus, and by Boot (1956) (34) who showed that subcutaneous ovarian grafts can maintain a pregnancy begun by transferring fertilized ova into pseudopregnant spayed hosts.

In primates and man, the onset of cyclic menstrual periods forms a satisfactory criterion of a successful graft (35, 36). Vaginal smears, estimates of steroid hormones or of gonadotropin in the urine, together with subjective impressions of alterations in the intensity of menopausal symptoms, can also be used in women (20–28, 37). Tuffier (1915) (35) and Bell (1925) (24) say that it takes three to four months for an ovarian graft to reestablish itself in women. Mandl and Zuckerman (1949) (35) report an interval of up to five months before cycles reappeared in monkeys carrying intraocular transplants, while Van Wagenen and Gardner (1953) (36) report the reappearance of menstruation about two months after intrasplenic grafting.

Other tests for the adequacy of hormone production are much simpler but usually show only that the graft is able to produce estrogen. This is especially true of rats and mice, where the estrous cycle can readily be studied by means of vaginal smears provided only that the animal's own ovaries have been removed and that no hormones are administered which might confuse the vaginal smear picture. In such instances one can record 1) the latent interval before the onset of the first postoperative estrous period, 2) the duration of positive response, 3) the proportion of animals giving a positive response, and 4) (in immature animals only) the time of vaginal opening and whether it is accelerated or not.

Occasionally the cyclical running activity of rats has been studied in special activity cages. Successful ovarian grafts restore the normal phasic activity in females and may even induce cyclic behavior in males (39).

The determination of the activity of ovarian grafts in the male usually depends on histologic examination of the graft. But an idea of the function of the graft can also be obtained by grafting a piece of vagina to provide a small pouch from which vaginal smears can be taken (40).

Considerable information is already available about the vaginal cycle response in spayed rodents after grafting. There is general agreement that autografts in rats first become active between six and twelve days later (41–45). Occasionally the interval may be a little shorter and in a few instances, especially those reported by May (1940) (46) who grafted newborn ovaries to the anterior chamber of the eye and not subcutaneously, the interval was 13 to 17 days.

The latent period is considerably increased if the graft has been frozen before transplantation. Thus Parkes and Smith (1953) (47), Green, Smith and Zuckerman (1950) (48) and Deanesly and Parkes (1957) (49) all report latent intervals about twice that for a normal graft. Such prolongation of the latent period had been reported as early as 1932 by Uprus (50) and again in 1942 by Payne and Meyer (51). Deanesly and Parkes (1957) (49) have recently described an extraordinary delay in the reappearance of estrogenic activity when grafts were frozen and thawed under conditions that were not optimal. It had been their impression that an animal would show estrous cycles within four weeks of grafting or not at all. Their later figures, for a composite group of grafts preserved under unfavorable conditions, showed that within one month of grafting only 16 out of 69 were active, at three months 30 out of 68, and at six to eight months 63 out of the 67 surviving animals were showing estrous changes in the vagina.

The latent period before estrus can also be used to distinguish autografts from homografts, according to Harris and Eakin (1949) (41). Intrastrain grafts took 13 to 14 days to produce the first postoperative estrus, as opposed to 8.0 days for the autografts and 24 days for homografts. In Parkes' (1950) (45) experiments, however, the prolongation of the latent period for homografts was not statistically significant.

Satisfactory grafts can apparently live for as long as a normal ovary, though their production of hormones may be slightly disturbed, as judged by the appearance of prolonged estrus or of rather longer cycles than normal. Mühlbock and Boot (1950) (52) report that subcutaneous grafts in mice may function for eighteen to twenty months but that irregularities in the cycle appear earlier

than normal. Much may depend on the amount of permanent damage that is done to the graft during transplantation.

So far it has been assumed that the process of grafting may either diminish the normal function of a graft, probably by the immediate destruction of tissues which occurs in the first few days after grafting, or may alter the sort of hormone produced according to the site of the graft (see p 411) Little and his colleagues (1951) (53) have shown in addition, however that the ovaries of CBA mice become permanently less able to produce ova if they are transplanted into the abnormal environment of the spleen, even though they are retransplanted subsequently to a normal environment. The grafts continue to produce estrogen and offspring which may be born after retransplantation show no signs of any somatic or genetic modifications which might have been induced. Transplantation *per se* may also increase the chance that a tumor will later develop in the ovary (54)

**Development of Ovarian Autograft**

A characteristic early feature of many subcutaneous grafts is the rapid formation of a new bursa around the ovary presumably by proliferation of the so-called germinal epithelium, which has been reported by Athias (1920) (55) to be very active three to eight days after grafting (see also Tamura, (1926) (56)) It should be mentioned here that the germinal epithelium often disappears without any obvious detriment to the total numbers of oöcytes remaining in the ovary an observation which throws doubt on the proposition that the epithelium is truly germinal and oögenetic (35) Butcher (1947) (57) believed that decapsulation of the young rat ovary may slow down its development and affect its behavior on transplantation Gaillard (1953) (58) also finds that the bursa is rapidly regenerated and that the final success of a transplant depends on the adequacy of this restoration.

Harris and Eakin (1949) (44) have described the histologic changes which occur in grafted ovaries in rats at various time intervals after grafting After twenty-four hours the grafts are avascular and already show a large central mass of necrotic elements surrounded by a rim of living tissue. The two zones have become more distinct in three days time and the host blood vessels are already linking up with the preexisting vessels in the graft, many of which however have been destroyed The central necrotic mass is largely replaced by macrophage elements within a week or two and finally disappears, leaving only a small scar Further follicular development and the formation of corpora lutea occur as a result of the growth of the viable fringe of the graft. Similar observations have been made for both subcutaneous and intracapsular grafts in mice by Jones and Krohn (1957) (59)

Deanesly (1956) (32) has described the histologic appearance of long term grafts in which ovulation had occurred and normal corpora lutea had been formed. Parkes (1958) (45) reports that all the usual normal structures were found in autografts surviving for a year or more. The frequent presence of follicular and lutein cysts, associated with persistent vaginal cornification is a reminder of the ease with which hypophyseal/ovarian relations can be unbalanced by the act of grafting Unfrozen glycerol-treated ovaries behave in a comparable fashion (Parkes and Smith 1953 (47))

Biskind and Biskind (1949) (60) were unable to find any recognizable surviving tissue 24 48 or 72 hours after intrasplenic transplantation Since follicles of all sizes are quite obvious later on Biskind and Biskind believed that they develop anew from proliferating germinal epithelial elements

The view based on similar evidence that neoformation of oöcytes may occur in frozen/thawed grafts (see p 413) has not been accepted by Green and coworkers (1956) (48), who point out that even under the best conditions of grafting, a large proportion of the oöcytes will be destroyed by the act of transplantation the proportion is certainly increased by preservation and freezing. This belief is confirmed by observations of Jones and Krohn (1937) (59) on the number of oöcytes remaining in intracapsular grafts at various intervals after grafting Even in the most successful grafts approximately half the total population of oöcytes was lost but there is always a rim of surviving tissue and it is in this zone that most primordial follicles are ordinarily to be found. Since the number of oöcytes seems to bear no relation to the presence or absence of a germinal epithelium one can only presume that, if neoformation of oöcytes ever occurs the germinal epithelium is not the component of the ovary which is responsible. It seems simpler and more

reasonable to reach the conclusion that neoformation does not take place—at any rate when the immediate postpartum period is ended. (For reviews of this perennial topic see Zuckerman, 1951 and 1956 (61, 62).)

*Transplantation can also be used to study the behavior of various components of endocrine organs in isolation.* Such experiments using transplants of α- and β-cell rich portions of anterior pituitary tissue and of the glomerulosa or reticularis zones of the adrenal cortex will be referred to later. The method has been applied to the ovary by Ingram (1957) (63) who has investigated whether the medullary tissue of the ovary (freed from the overlying follicle-containing cortical tissue) contributes anything to the process of oögenesis in rabbits. Pieces of interstitial tissue uncontaminated with follicles can easily be prepared from the medulla of the rabbit's ovary and were grafted both subcutaneously and to the anterior chamber of the eye. The experiments provided no evidence to support the old notion that these cells are precursors of oöcytes. Even when stimulated with injections of chorionic gonadotropin the grafts gradually became smaller and did not survive indefinitely.

*One of the purposes of ovarian transplantation* is to show that the gland functions normally after the destruction of its normal nerve supply. It is therefore rather surprising to find, according to Goecke and Beaufays (1930) (64), that numerous fine autonomic nerve fibres which can be found in the normal ovary and which degenerate in the first week after grafting soon start to regenerate.

## Development of Ovarian Homograft

The cellular reactions to an ovarian homograft do not seem to differ in any particular way from those which characterize the response to homografts of other organs (figs. 178–183). According to Harris and Eakin (1949) (44) there is no histologic evidence of any local response until five to seven days after grafting, when lymphocytes begin to infiltrate the graft. Within two to three weeks all trace of organized ovarian tissue is lost, the lymphocytes are replaced with fibrous connective tissue and sometimes plasma cells, and the remaining dead tissue is cleared up by fatty phagocytic cells with a foamy cytoplasm that may cause them to be mistaken for luteal cells. Harris and Eakin (1949) (44) consider that the lymphocytic reaction is not so intense in hosts which have been spayed (see p. 410).

In instances where the immune response is not maximal, healthy looking small oöcytes may be seen lying in a fibrous stroma and surrounded with infiltrating lymphocytes. The ovary may therefore survive and continue to function at least partially under the conditions of a niggling ineffective homograft reaction such as may cause the appearance of scales, eczematous patches and small scabs on the surface of a skin graft which is not fully compatible with the host.

## Sites for Transplanting Ovary

Investigators have been catholic in their choice of sites for transplantation. A wide variety of positions in the muscles and subcutaneous tissues and various organs have been used at one time or another. Only those sites of particular interest will be discussed here.

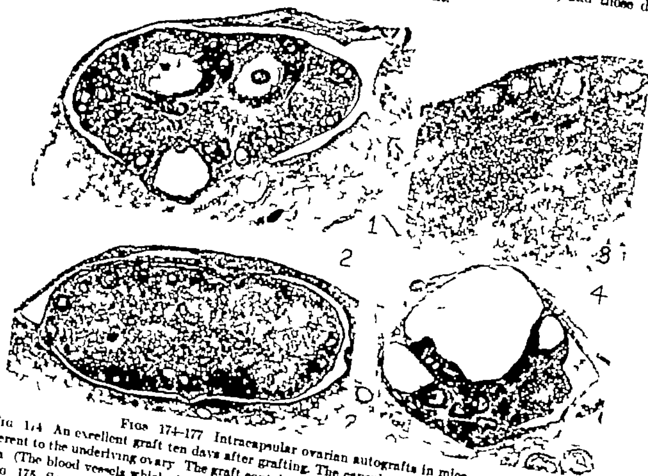
### *Orthotopic Transplantation*

The two very different functions which the ovary has to subserve make a full assessment of the results of transplantation difficult. From the viewpoint of the production of ovarian hormones *it is usually regarded as sufficient to demonstrate* the appearance of regular estrous cycles after grafting the ovary into a spayed host. In fact, this is only evidence that the grafts can produce estrogen but it does have the advantage that it is a simple test and allows the graft to be made anywhere in the body. If the production of fertilized ova is required, the graft must be made orthotopically so that any ova which are ovulated have an opportunity to pass into the Fallopian tubes and then into the uterus.

In the early 1900s many attempts were made to sew the ovaries of guinea pigs and rabbits on to the ovarian ligaments but without any great success. More recently Robertson (1940, 1942 and 1945) (65–67) has reinvestigated the possibility of carrying out orthotopic transplantation in mice, a species especially adapted to such studies because a bursa or capsule completely surrounds the ovary and can form a covering for the graft. Robertson exchanged ovaries between two strains of mice which differed only in a single visible gene-controlled character and was able to show that litters were born which contained offspring necessarily derived from the donor ovary. Success was achieved only when the host's own

ovaries were completely removed. The general method, as developed at Bar Harbor is described by Russell and Hurst (1945) (68) who laid out in detail the ways in which genetic color markers can provide complete evidence that the offspring must have come from the graft and not from regenerated ovarian tissue. Stevens (1955) (69) using this technique reports that out of 24 animals which were bilaterally spayed and then grafted, 16 became pregnant and had a total of 110 litters with 381 young. His other experiments, which related to the validity of Halsted's law are discussed on p 417 In my own hands the success rate has not been so high. (See figures 174–177 for representative sections of orthotopic autotransplants.)

The method of orthotopic transplantation has also been used by Russell and Douglass (1945) (70) to obtain offspring from embryonic mouse ovaries. Donor ovaries were obtained from 13½ to 14½-day-old embryos and in three experiments 3 4 and 10 young were produced. Embryonic ovaries have also been used by Russell and Gower (1950) (71) and by Russell, Murray Small, and Silvers (1954) (72) to distinguish between effects due to the expression of deficient genes (*e.g.*, SpSp and Wv Wv) and those due to environment.




FIGS 174–177 Intracapsular ovarian autografts in mice

FIG 174 An excellent graft ten days after grafting. The capsule is perfectly reformed and nowhere adherent to the underlying ovary The graft contains numerous follicles of all sizes and recent corpora lutea (The blood vessels which show up black were perfused with India ink ) X30

FIG 175 Some of the difficulties encountered in intracapsular ovarian grafts are shown in this ovary three days after grafting Some adhesions between the capsule and the surface of the ovary are already evident But the main feature is the large central area of necrosis There is only a narrow band of living tissue which form a fringe of viable oöcytes X34

FIG 176 High power view of part of a graft removed one day after grafting Note the viable fringe of follicles and the beginning of dissolution of the rest of the tissue X80

FIG 177 A graft ten days after grafting. The graft has been distorted by the development of cysts within the capsule Note the recent corpora lutea and the three ova which have been ovulated into one of the cysts without any possibility of reaching the intact coils of Fallopian tube seen at the bottom of the photograph X2_

A modification of the method has been proposed by *Parrott and Parkes (1957) (73)* which avoids the difficulties introduced by having to remove the host's ovaries from the capsule. Instead they destroy the ovaries by irradiation and then implant the graft ovaries direct into the capsule.

Orthotopic transplants between two breeds of dog (74) and between rats and mice (75, 76) have been reported but, though the hosts are said to have shown periods of estrus, they did not become pregnant.

Another way of achieving pregnancies from grafted ovaries would be by placing the ovary directly into the uterus and, according to Wiesner (1923) (77), such experiments succeeded in two out of seven autografts and in fourteen out of sixty-eight homografts. The half ovary was simply inserted through a small hole into the top of the uterine horn of recently postpartum rats. Weber (1927) (78) confirmed Wiesner's observations and succeeded in about 50 per cent of cases. *McLaren and Michie (1955) (79)* using mice could not repeat Wiesner's work on rats. The grafts took but were never retained within the uterus. Heitmayer (1934) (80), who transferred rabbit's ovary to the uterine wall by means of a pedicle graft, was successful inasmuch as he found corpora lutea in the grafts and ova within the cavity of the uterus, but the rabbits never became pregnant despite this absolute proof of ovulation.

Wiesner's and Weber's demonstration, if it is confirmed, that ova released from intra-uterine grafts are fertilized and may implant, raises an interesting point as to the normal function of the Fallopian tubes. It is usually believed (and the failure of Heitmayer's rabbits to become pregnant supports this belief) that slow passage of ova through the tubes is necessary to allow uterine conditions appropriate to pregnancy to develop and that fertilized eggs whose passage into the uterus is accelerated (*e.g.* by hormonal treatment) fail to implant.

*Anterior Chamber of the Eye*

The obvious and important advantage of this site is that it permits the repeated observations to be made which are necessary for a study of the evolution and response to stimuli of grafts. A general survey of the value of the eye for the transplantation of embryonic grafts of all endocrine tissues, including the ovary, is given by Dameron (1951) (81). Schochet (1920) (82) seems to have been the first to use this site for transplants of the ovary in rats and reported that eight weeks later ova could be seen lying free in the anterior chamber. *Similar grafts in rabbits* behave normally; they ovulate and form corpora lutea in response to mating or the injection of chorionic gonadotrophin (83–85). The grafts remained inactive and unresponsive, however, if one ovary was left in the pelvis. These workers therefore carried out the type of experiment reported later by Lane and Markee *(1941) (86)*, *which is taken to indicate that there is preferential uptake* of gonadotropin by an ovary whose normal blood supply has not been disturbed.

Since rabbit grafts will also respond to injections of pregnancy urine, the intraocular graft can be used as a modified Friedman test for pregnancy (83, 87, 88).

The anterior chamber of the eye is one of the sites which are usually regarded as immunologically privileged in the sense that homografts to the anterior chamber more often survive than would be expected of homografts elsewhere. According to Medawar (1948) (89), working on skin grafts in rabbits, this privilege exists only while the grafts remain unvascularised, but according to Woodruff and Woodruff (1950) (90) it remains true of thyroid grafts even if vascularization occurs (see p. 415). Many of those who have reported successful ovarian homografting in rodents have used this site for the graft, a fact which perhaps further complicates the interpretation of the many contradictory observations that have been made. In rabbits, for example, Ward, Gardner and Newton (1953) (91) report that 78.4 per cent of 134 homologous grafts remained viable and would respond to the injection of gonadotropin. It is noteworthy that these workers found that homologous takes were highest in non-spayed females. Success was less common in spayed females than in castrated males and unilaterally spayed animals were more tolerant than completely spayed rabbits. Such observations run counter to the generally accepted findings.

A further difference from the normal run of expectation is that the youngest grafts (newborn to four weeks old) were less successful than 6- and 12-week-old grafts.

*Spleen and Other Organs Draining into Portal Circulation*

Ovaries seem to have been first transplanted into the spleen by Long and Evans (1922) (41) who reported the failure of such grafts to induce estrous cycles in the hosts. In the light of more recent observations detailed below their observations have taken on an added significance which was not recognized at the time.

Golden and Severinghaus (1938) (92) took the important step when they compared the responses to grafts in the axilla with those to grafts in the mesentery. Spayed hosts with grafts in the mesentery, the blood from which drained to the liver, did not show estrous cycles. Cycles began when the grafts were transferred from the abdomen to the axilla. The evident explanation that the liver cells inactivated the estrogen in the blood during its passage through the portal circulation was put forward by Golden and Severinghaus, though Pfeiffer (1936) (93) had realised earlier that drainage through the portal system might affect the sort of hormonal stimulus to the pituitary which a graft would provide. Later Biskind and Biskind (1944) (94) found that estrous cycles occur in rats with intrasplenic grafts only when the grafts become revascularised by adhesions to the abdominal wall, through which the blood can drain direct into the systemic circulation and not *via* the portal system. There is nothing about the spleen *per se* which interferes with the function of a graft, since transplantation to other abdominal organs, such as the pancreas, gives the same result (95). What matters is that the blood should drain directly to the liver.

It was thought at first that the inactivation of estrogen by the liver in rats was complete but this is certainly not so in other species and some hormone probably always escapes destruction even in rats. Bernstorf (1951) (96) showed clearly that spayed mice with intrasplenic grafts were not physiological castrates since, although the vaginal epithelium was not usually cornified, the uteri weighed considerably more than they do in spayed animals. With even this amount of leakage of estrogen the grafts were about six times heavier than normal, and Miller and Pfeiffer (1950) (97) by means of parabiosis experiments, confirmed the obvious inference that increased amounts of gonadotropin were circulating. Similar observations have been made in guinea pigs by Wenner and Hofmann (1950) (98), in the rabbit by Peckham and Greene (1952) (99), and in the hamster where the changes are much less pronounced, by Takewaki (1955) (100).

Hepatic inactivation is least evident in monkeys. All the normal signs of estrogenic activity (coloration and swelling of the sex skin, vaginal desquamation) and menstrual cycles continue after intrasplenic transplantation of only half of one ovary (36, 101).

Other steroid hormones are not affected by passage through the liver to the same extent as estrogen. Kullander (1954 and 1956) (102, 103) found that the secondary sex organs of rats which had received intrasplenic ovarian transplants when three weeks old showed signs of stimulation which he believed were the consequence of the production of amounts of progesterone that, unlike estrogen, passed through the liver unharmed. Kullander (1956) (33) also induced deciduomata in such rats, another piece of evidence that the grafts were producing progesterone.

Considerable work has been carried out on the histologic changes in intrasplenic grafts. Deane and Fawcett (1950) (104) have applied histochemical methods to a series of ovarian grafts to the spleen and compared the findings with the results of grafting to the kidney and liver of spayed rats. Grafts to the kidney showed a normal appearance with numerous follicles and active corpora lutea but scanty interstitial tissue. So too did grafts in the liver. Apparently the estrogen produced by these latter grafts could escape destruction since normal estrous cycles continued after grafting. The intrasplenic grafts appeared very different. Within a month they contained far fewer follicles, within two months there were practically no follicles at all and the expanding mass of the graft consisted of corpora lutea. Subsequently the structure became more and more disorganized. At first, there were large areas of granulosa cell proliferations, which were actively secreting estrogen; later luteomatous tissue reappeared. Comparable changes in mice have also been described (105, 106).

The most characteristic and important fact about intrasplenic grafts is the development in them of tumors (60) which resemble in many respects those which appear after x-irradiation of the ovary. The unrestrained spread of undifferentiated granulosa cells and formation of tumors

begin about 12 weeks after grafting when most of the follicles and oöcytes have disappeared. Development of tumors takes longer in rats than in mice (94) and in guinea pigs may take up to 12 months (107). In this species the proliferative changes usually occur in luteal tissue (with the formation of luteomata after earlier hemorrhagic follicles) but granulosa-cell tumors have occurred five years after transplantation (108). Iglesias, Mardones and *Lipschütz (1953) (109)* have studied the evolution of luteomata in guinea pigs over a period of up to three years. The tumors did not metastasize and could not be transplanted to another guinea pig, though the granulosa-cell tumors which finally developed in one guinea pig five years after grafting did metastasize to the liver (108).

If only one ovary is transplanted and the other is allowed to remain in its normal position, the intrasplenic graft remains entirely inactive (110–112) but is apparently capable of responding to the condition of pregnancy by active growth of follicles. As soon, however, as the remaining normal ovary is removed there is immediate rapid development in the graft even as long as 465 days after the original grafting (111) and before long the proliferation reaches such an extent that the characteristic granulosa and luteal cell tumors develop. Exogenous gonadotropin can also speed up the development of the luteomata and granulosa cell tumors (113), while conversely injections of antigonadotropin can suppress the growth of the transplant (114).

The explanation for these changes is thought to lie in the fact that the pituitary of the host is relieved of the curb to its release of gonadotropins normally provided by circulating estrogen and proceeds to secrete large quantities without restraint. Under constant stimulation from this gonadotropin the transplant develops tumors which may become fully autonomous and malignant (115, 116) but which for a time at least fall into the group known as hormone-dependent tumors. *According to Gardner (1955) (117)* some of the tumors which may develop in mice are transplantable and several of them are hormone-producing. He believes that they are all derived from the germinal epithelium of the graft.

It is well known that, as ovarian function declines in women at the menopause, the level of circulating gonadotropin rises. On the basis of animal experiments one would therefore expect an increased incidence of ovarian tumors in women at about this time, but nothing of the sort is found. To explain this discrepancy it could be assumed that the ovaries of old animals become refractory to any *stimulation. But if such old* tissue is grafted into the spleen of a young mouse it develops granulosa-cell tumors almost as readily as does a young ovary (118, 119).

Intrasplenic ovarian tumors did not develop in hypophysectomized rats even though they were given continuous treatment with gonadotropins (120). The essential component of the pituitary's contribution to tumorigenesis must therefore be sought elsewhere. A complete absence of systemic estrogen is not *essential, for, however the effect* may be mediated, some tumors also developed in animals who continued to show estrous cycles after grafting. However, the control old rats used by Bøe and associates (1954) (120) also failed to produce tumors during a period of observation that was restricted to five months. The failure of the *prolonged treatment with injected gonado*tropins may be attributed as well to the development of antigonadotropic substances as to lack of responsiveness of the end-organ.

Comparisons between the responses of human and rodent ovaries to prolonged pituitary stimulation must be made with great care, for the two species do not seem to have equivalent postmenopausal periods. It is by no means certain that the mouse ovary undergoes menopausal changes like those which occur in women, and the ovaries of mice which are no longer capable of breeding still look healthy and active, and secrete estrogen for at least some time. Their stromal and granulosal elements may therefore respond in ways which the cirrhotic fibrous tissue remaining in the human ovary would be unable to copy. Transplantation of the human ovary to the spleen has been used in the treatment of hopeless malignancy, with reported advantage to the patients (121). The final histologic appearance of such grafts will be of great interest when reports become available.

*The use of the spleen as a site for transplanting* endocrine organs has therefore made an important contribution in the field of tumorigenesis for it has demonstrated beyond question the importance of hormonal factors in the onset of cancerous changes.

Intrasplenic grafts have been used with great success (especially by Lipschütz and his colleagues) in other fields more closely related to endocrinology, to disentangle the direct from

the indirect effects of steroid hormones on the ovary and for further investigation of the hypophyseal-ovarian balance Thus, it can be shown that minute amounts of estrogen implanted subcutaneously inhibit the appearance of hemorrhagic follicles and stimulate luteinization in guinea pigs. Since the hormone is ineffective when implanted near to the intrasplenic graft, where it could only have a direct effect, the action must be an indirect one on the pituitary (122, 123) Progesterone or desoxycorticosterone can counteract this action on luteinization but not on follicular growth (107) The further possibility that degradation products of estrogen derived from the liver's metabolic activities may affect the pituitary and thus modify the behavior of the transplant, is discussed by Achilles and Sturgis (1951) (124)

## Effect of Site of Transplantation on Secretion of Hormones by the Graft

The position of the graft influences not only the development of the graft itself but also the sorts of hormones that are secreted

Hill (1937) (125, 126) first showed that ovaries transplanted subcutaneously to the ears of castrated male mice secrete sufficient male hormone to maintain the seminal vesicles and prostate in a normal condition for at least 100 days Hill (1941) (127) allowed grafts applied to the ears of mice to remain for up to 605 days. *Production of androgen was sufficient to maintain normal-sized seminal vesicles for 6 months after grafting but had ceased after 405 days, though estrogen was still being secreted.* Production of male hormone was increased by cooling the environment to 22°C but stopped if the temperature was increased to 33°C Deanesly (1938) (128) confirmed this finding in the rat but related hormone production to luteinization of the theca interna of the follicles rather than to ambient temperature changes. The grafts continued to produce estrogen. Ovaries grafted into the ears of female mice (but not intraperitoneally nor subcutaneously) would also maintain a healthy epithelium in transplants of seminal vesicle made at the same time.

Lampton and Miller (1940 1941) (129 130) agreed that male and female rats with ovarian grafts in the ear produce more androgen when kept at 22°C than at 33°C but pointed out that the amount was always less than that produced by a normal male. Restoration of the male accessory organs was incomplete even in the cool environment.

No histologic feature in the ovary can be correlated for certain with the changes in hormone production which, on the basis of the conditions of the secondary sex organs, can best be reproduced by a mixture of progesterone and androgen (131) Hernandez (1943) (132) like Deanesly (1938) (128) believes that androgenic activity is closely associated with an increase in the number of theca cells. In his rats, increased production of androgen from grafts in the tail was associated with constant vaginal cornification Ovarian grafts to the ears stimulated growth of the prostatic rudiments found in the females of a special strain of rats used by Witschi, Mahoney, and Riley (1938) (133) but only if the grafts were luteinized and the vaginal cycles were irregular There was no growth if there was follicular development and normal estrous cycles Bielschowsky and Hall (1953) (134) found that autotransplants to the tail of rats did not form corpora lutea. The grafts produced only small quantities of estrogen and appreciable amounts of androgen

Katsh (1950) (135) also appears to think that luteinization of the follicles is involved in the response. By grafting pieces of ovary directly onto the seminal vesicle of castrates he was able to demonstrate the production of male hormone by the ovary even at the temperature of the abdomen. Using a similar technique Takewaki (1953) (136) showed that intrasplenic, but not subcutaneous ovarian grafts can produce androgen.

Idzkowsky and Starkey (1942) (137) confirmed Katsh's findings and showed, in addition, that the adrenal X zone is reduced or disappears, a fact which can be attributed to the production of androgen. They also succeeded where Hill and Deanesly had failed in stimulating the grafts to produce more androgen by injecting pregnant mare serum (PMS) though treatment did not increase the amount of lutein tissue Treatment with PMS did not influence the X-zone in the absence of grafts Zizine and Zizine (1952) (138) reported on the contrary that the X zone in the adrenal cortex increased in size and considered that the grafts could not be secreting androgen.

Masculinizing tumors of the ovary are not uncommon, and the male gonad certainly produces estrogen as well as androgen. We need not

therefore be surprised at the capacity of the ovary to produce so-called androgen under abnormal circumstances, though the factor of transplantation clearly may have very marked effects on the performance of the gland. No other comparable temperature effects have been noted for other endocrine glands. The cryptorchid testis may produce less androgen than normal after a long time has elapsed, and certainly spermatogenesis stops, but there is no evidence that the graft begins to produce extra estrogen, nor does the hormone production of grafted adrenal cortical tissue vary with the site of grafting.

The close embryologic origin and histologic similarities of adrenal cortex and some components of the ovary have been mirrored in the way each gland can sometimes take over the hormone production of the other. Hill (1944) (139) found that ovarian grafts in the ears of castrated male mice enabled them to survive adrenalectomy, and in 1948 (140) he extended the observation to cover adrenalectomized female mice as well. They died as soon as the grafts were removed. Later Hill (1949) (141) showed that cutting the ovarian nerves and artery would also enable mice to survive adrenalectomy, but he provides no explanation for this rather remarkable finding.

The ovary's ability to take over adrenal cortical function cannot, for certain, be related to a temperature effect. Although Bernstorf and Hill (1953) (142) were unable to show that an ovarian autograft in the spleen of either immature or young adult female rats permitted the hosts to survive adrenalectomy for a significant period beyond the survival time of adrenalectomized control animals, Lichton and associates (1953) (143), on the other hand, found that 28 per cent of rats with transplants in the spleen (and 20 per cent with transplants in the ear) survived adrenalectomy for 75 days or more. The ovaries of those animals which survived contain abundant luteal tissue not organized within corpora lutea. In those which died within 30 days, the luteal tissue was mainly restricted to the corpora lutea. The ear grafts were more successful than the spleen grafts in protecting the surviving animals against the stress of histamine poisoning. Zizine (1954) (144) has also shown that rats of both sexes with an ovarian graft in the ear remain alive after adrenalectomy. Liver glycogen levels were lower than in normal rats but higher than in adrenalectomized animals which did not receive grafts.

## Preservation of Ovarian Tissue

Tuffier (1911) (25) attempted without success to preserve human material in a refrigerator for periods of from one hour to 46 days. In 1928 Lipschütz (145) managed to keep guinea pig ovaries alive for up to 10 days by storing them at just about freezing point. When grafted, the stored ovaries feminized male guinea pigs, though the latent period before changes occurred in the secondary sex organs of the host was longer than normal. Much of the ovarian tissue was destroyed, but histologically at least some of the follicles survived the treatment. In 1932 he reported (146) that such frozen grafts may survive and continue to produce endocrine effects for up to 2½ years. Lipschütz (1929) (147) also reported that partial drying to about 50 per cent loss of weight was compatible with the production of an endocrine effect when the tissue was grafted.

According to Uprus (1932) (50) temperatures lower than 0°C. destroyed the feminizing activity of ovarian grafts in male guinea pigs. Preservation at 0° to 10°C. for up to three days was successful in 50 per cent of trials though the grafts took longer than normal to become active.

Payne and Meyer (1942) (51) stored young rat ovaries at 10°C. and found that grafting was followed by estrous cycles in the hosts. Quick freezing in liquid air was not successful.

Interest in the possibility of preserving tissues at low temperatures increased greatly after wartime achievements in the preservation and storage of red blood cells. It soon became clear that sperm could readily be preserved if they were frozen in media which contained glycerol. Smith and Parkes (47, 148) applied this knowledge to the preservation of ovaries and found that the average time to establish an active graft varied with the medium in which the graft had been frozen; there was always some destruction of hormone producing tissue. A modified technique for preserving ovarian tissue by freezing a small number of relatively large pieces in a single cooler and storing in glycerol serum at −79°C. was more successful (149). The figures by Deanesly and Parkes (1957) (49) show that, even under optimal conditions, frozen thawed grafts take twice as long as normal to restore estrous cycles. If conditions are less satisfactory, the period may be three weeks or more (see also

p 405) Glycerol with horse serum media are much less damaging than those containing glycerol with saline only

There is no doubt that oöcytes in the ovary are more readily destroyed by the freezing and thawing process than are the hormone producing components of the ovary In the early work of Smith and Parkes (1951) (148) it appeared that all the oöcytes were destroyed, and their report (1953) (47) that healthy follicles had reappeared six days after grafting suggested to them that there might be new growth of oöcytes. This assumption necessarily depends on the evidence that the grafts contained no oöcytes when grafted, but Deanesly (1954) (150) has shown that this view cannot be sustained. Green and coworkers (1956) (48) in a further study autotransplanted pieces of rat ovarian tissue which had either been treated with glycerol/saline alone, or been treated and then frozen and thawed. The grafts were removed at intervals of 2 to 30 days after grafting, and the number of oöcytes in the grafts counted. Using for comparison, the number contained in control fragments which had not been grafted they found that, even without freezing, the number of oöcytes in a graft was at best only half the normal figure, a finding of importance when one is considering whether the grafted ovary can ever be regarded as an equivalent of a normal ovary They could find no evidence of reformation of oöcytes in the frozen grafts. The number of oöcytes varied widely between grafts but the variations were entirely independent of the length of time which had elapsed since grafting.

**Fate of Ovarian Homografts and Heterografts**

No better start for a section on the results of homografts of ovarian tissue can be made than by quoting Smith and Parkes (1951) (151)

> many experimental endocrinologists blissfully ignorant of immunological complications and with unlimited material at their disposal have made homografts of various tissues notably mammalian gonads with complete nonchalance and notable effect Steinach and Lipschütz and many others have grafted ovaries into male mammals testes into female mammals and both into both and have made observations on the resulting intersexuality without apparently being surprised that the grafts essentially homografts took persisted and functioned.

Steinach and Lipschütz indeed seem to have been exceptionally fortunate with their ovarian homografts though it is not always clear what proportion of the total of their experiments is represented by the successful grafts which they report in detail, nor how inbred their animals were. Other recent evidence bearing on the problem is detailed below

*Mice* Strong (1930) (152) and Nordholt (1940) (153), both of whom were careful to control the genetic incompatibility of host and donor by using different inbred strains of mice, failed to obtain successful grafts. Krohn (1955) (154) using both vaginal smear records and histologic observations to judge the condition of the grafts, came to the same conclusion.

Parkes (1956) (155) has studied the effect of exchanging ovaries between pairs from five different strains of mice. Forty per cent or more of the grafts "took' and produced at least one period of estrus in eight of the sixteen combinations of strain that he tried. In the other eight combinations there were no temporarily successful grafts at all, and only 6 out of the total of 152 animals studied showed any signs of function four weeks after grafting.

Ferguson and Kirschbaum (1954) (156) who wished primarily to see whether injections of gonadotropin would prolong the survival of the graft (see p 417) report that grafts of ovaries from C57BL to A strain mice are ordinarily destroyed in three weeks or less. Since they used castrated males as hosts they had to rely entirely on histologic appearance to decide whether the grafts survived or not. Russell and Hurst (1945) (68) quoting unpublished work of Robertson and of Kellog say that interstrain transplants were unsuccessful in a total of 59 experiments.

In view of the suggestion by Billingham, Brent, Medawar and Sparrow (1954) (157) that inbred lines of mice within a strain might very soon diverge so that minor incompatibilities between host and donor became apparent, it is interesting to note that two separate lines of A strain mice maintained by Huseby and Bittner (1951) (158) for more than 50 generations would still readily accept grafts in exchange

*Rats* The most extensive series of observations so far made on rats is reported in a paper by Harris and Eakin (1949) (44) Besides autografts they studied sibling, intrastrain interstrain and heterografts (mouse to rat) Vaginal smears were taken daily and histologic observations made on

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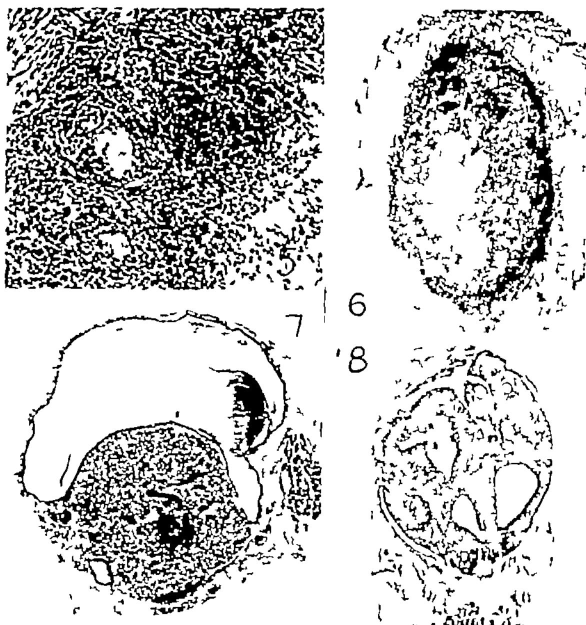
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FIGS. 178–183 The reaction to ovarian homografts in rodents

FIG. 178 An interstrain graft (mouse) six days after grafting. Proliferating germinal epithelial cells survive together with clumps of granulosa cell and an occasional recognizable follicle. Round cell infiltration has begun. ×80

FIG. 179 An interstrain homograft (mouse) twelve days after grafting. Intense infiltration with lymphocytes and total loss of organized ovarian tissue. ×27

FIG. 180 An interstrain homograft (mouse) fifteen days after grafting. Note the attempt at reformation of a capsule. The structure of the graft is totally destroyed and infiltrated with round cell. ×?1

FIG. 181 A 30 day old autograft in a mouse for comparison with figures 179 and 180. ×?

tissue recovered at autopsy (usually 30 days but sometimes 3 months later). Some of their results which are based on groups of 15 to 4? experiments are summarized in table 8. Two strains of rats were used. One had been inbred "to some extent" for over 3? generations and was used for the sibling and intra strain grafts. The other stock was from a dealer and had not been inbred at all. Exchanges were carried out reciprocally in the series of homografts but no distinction between the two host-donor combination is made in presenting the results.

The figures (table 8) clearly indicate that the expectation of success diminishes with increasing genetic disparity and Harris and Eakin conclude. That the homografts at least would all even-



FIG 182 Intracapsular interstrain homograft (mouse) 42 days after grafting Inflammatory reaction has died down and the remnants of the graft are being removed by fat containing phagocytic cells ×106

FIG 183 Intense infiltration with round cells in a rat homograft 21 days after grafting The host had been unilaterally ovariectomized ×106

tually succumb to the unfavorable environment seems probable to us but it is entirely possible that ovarian grafts more closely related genetically to their hosts might in some instances survive indefinitely The proportion of successful intrastrain grafts and sibling grafts can be taken as a rough measure of the degree of genetic homogeneity that had been achieved in the partially inbred strain. Even where homografts survived for the period of the experiment the cycles were less regular and the periods of cornification more prolonged than with autografts.

Experiments reported by Ingram and Krohn (1950) (160) refer to grafts both within an incompletely inbred strain and also between strains Their criterion of a surviving graft was based on the presence of any quantity however small, of living graft tissue as judged histologically 21 days after grafting. They came to the general conclusion that the grafts would not have survived indefinitely but that the exact time of survival could be modified by the conditions of the experiment. As in Harris and Eakin s earlier work, the results varied with genetic disparity between host and donor but the proportion of surviving grafts when the disparity was greatest was less than Harris and Eakin found

The other main papers directed particularly

TABLE 8

*Percentage success with different forms of ovarian grafts in rats (from Harris and Eakin (1949) (44))*

| Type of Transplant | Follicles and Corpora Lutea Present | Hosts with Recurrent Vaginal Cornification | Latent Interval before Cornification |
|---|---|---|---|
| | % | % | days |
| Autograft | 90 0 | 92 8 | 8 0 ± 4 9 |
| Sibling graft | 61 9 | 81 6 | 14 0 ± 5 5 |
| Intrastrain graft | 54 2 | 80 0 | 13 3 ± 6 1 |
| Homograft | 25 9 | 35 7 | 24 1 ± 0 7 |
| Heterograft | 0 | 0 | |

at the immunologic and not the endocrinologic consequences of ovarian grafting in rats are those by Billingham and Parkes (1955) (160) and Parkes (1950) (45). In these experiments two strains of rat were employed—one hooded, the other albino—which had been closed and separate (but not inbred) colonies for many years. The results of grafting within each strain need indicate no more than that partial genetic homogeneity had occurred. Of more immediate interest, however, are the figures for the interstrain grafts (Parkes, 1950) (45). Here two out of eight spayed albino hosts which had received ovaries from hooded rats were still displaying vaginal cycles 12 to 13 months after grafting, while the proportion for the reciprocal graft was five out of ten, an extremely and unexpectedly high level of survival. Persistent cornification was a noticeable feature. Histologically, there was no clear correlation between the condition of the graft and the relationship between host and donor.

In discussing these results Parkes says:

> 'The results described above show that in the strains of rats used, intrastrain homografts are slightly less likely to become established than autografts, and interstrain homografts less likely than intrastrain homografts. The differences, however, were nothing like so great as is commonly supposed, and either the two strains of rats used are exceptional or else some of the previous work on interstrain homografting is in need of amplification.'

Parkes emphasizes the fact that grafts are either destroyed early (within a few weeks) or persist indefinitely, a view which is not in line with the evidence for the late break-down of skin homografts when the genetic disparity between host and donor is only slight (161).

The histologic condition of these grafts and of autografts (Parkes' material) has been further studied by Deanesly (1956) (32). The intrastrain homografts showed a wide range of lymphocytic response, which did not necessarily interfere with the growth and development of follicles and corpora lutea. Small primordial follicles persisted even in heavily infiltrated tissue. Those graft with numerous lymphocytes were less likely to survive when restored to their original environment.

*Rabbits.* Hannan (1929) (162) reported that in twenty experiments on rabbits follicles were still numerous in homografts at the end of a fortnight. However, all traces of ovarian tissue disappeared in the next 6 weeks and none of the animals showed any estrous behavior.

*Dogs.* Whitney (1946) (74) twice transferred the ovaries from a bloodhound to a foxhound. The dogs came into estrus twice and mated without becoming pregnant. Seventy days after the second estrus the grafted ovaries were said to contain corpora lutea and innumerable follicles greatly exceeding the number to be expected in a dog of the host's age.

*The Survival of Heterografts*

It is almost universally agreed that heterografts are rapidly destroyed (Pettinari, 1928) (31). Turner (1937) (163), for example, reports that ovaries (and testes) from young mice always elicited and succumbed to a violent lymphocytic reaction soon after transfer to the anterior chamber of the eye of either male or female gonadectomized rats.

Human ovarian grafts failed when transferred to monkeys according to Hannan (1931) (164).

On the other hand, heterografts between mice and rats have been successful according to Saunders (1940) (76), who believes that mouse to rat grafts succeed more readily because the larger rat's pituitary can better maintain the smaller animal's gonad than vice versa. Pincus (1931) (75), too, thought that grafts from mice to rats might survive long enough to provide estrous cycles but that ultimately they become infiltrated and fibrotic. There seems to be some doubt about the completeness of his ovariectomies.

*Mitigation of the Homograft Reaction*

From the observations described above it may be concluded that the ovary of many species behaves in accordance with the general laws of transplantation immunity. On the other hand, interstrain grafts in rats sometimes seem more able to survive than might be expected. But in this particular laboratory animal one usually has neither the complete lack of inbreeding of many other species nor the completely inbred strains of mice. One might therefore expect to find all gradations of reactivity to a foreign graft—as indeed one does.

But in addition there is good evidence that ovarian grafts in rats are not destroyed as quickly as skin grafts from the same donor (160).

though the fact that the development of an immunity to an earlier skin graft will accelerate the destruction of a subsequent ovarian graft indicates that we are dealing with a mechanism that is fundamentally of an immunologic kind. We may therefore, agree that endocrine organs, as exemplified for the present by the ovary are not absolutely privileged from immunologic assault but that the violence of the assault may sometimes be abated and resistance to it protracted.

There are many factors which must be taken into account in discussing this problem. See Harris and Eakin (1949) (44) Krohn (1955) (154) Billingham and Parkes (1955) (160) Parkes (1956) (155, 45) Ingram and Krohn (1956) (159)

Of these the role of the "physiological need of the organism" as set out in Halsted's law is perhaps the oldest and most talked about (456) According to him endocrine tissues can be grafted successfully only when there is an absolute deficiency of their secretions in the host (i.e. in bilaterally spayed animals, when discussing the ovary) Halsted intended this generalization to apply only to autografts it was extended to cover the behavior of homografts later but not by Halsted, who in fact, did not believe that homografts survived. At the time (1909) of course, it was impossible to translate this generalization into terms of pituitary hormones, which were still unknown But nowadays it is usual to say that the absence of the host's gonads stimulates the increased production of gonadotropin by the pituitary and that this, in turn, protects the graft. Bearing in mind that some endocrine glands (including the parathyroid, with which Halsted worked) do not seem to be controlled by pituitary trophic principles, we must now turn to the following questions

1) Do conditions of extra production of gonadotropin increase the likelihood that homografts will succeed?

2) If so how is this achieved?

The first point to deal with is the large body of evidence which seems to show that grafts are more successful in spayed animals than in intact animals. Such observations have been made by many workers. They do not, however entirely prove the point, for the distinctions between success and failure are often made on the basis of follicular growth and development rather than on the life or death of the graft. Extra gonadotropin will certainly stimulate growth of a graft (just as of an intact ovary) and would be expected to do so But grafts, even autografts, seem unable to trap or to make use of gonadotropin in the presence of an intact ovary (84 86) which, perhaps because of its better blood supply seems able to remove all gonadotropin preferentially even when extra is provided. Grafts in these circumstances are only dormant and not dead (43) That this is so is beautifully demonstrated by the work of Stevens (1955) (69) Using the color marker gene technique for the identification of offspring from orthotopic ovarian transplants, he showed that practically none of the mice born from mothers with one isografted and one normal ovary came from the graft. If however the normal remaining ovary was removed 6 to 7 months after the grafting the mice proceeded to give birth to litters from what had been the dormant ovary In any case, as Parkes (1956) (45) points out, many grafts are transplanted at the same time as the host's ovaries are removed, and there is, therefore, no time for increased pituitary activity to influence the initial taking of the graft. The experiments of Ingram and Krohn (1956) (159) also show that whatever advantage there may be in spaying becomes less and less as genetic diversity increases. Spaying increased the proportion of sib homografts which survived for 21 days but not that of full homografts.

Finally it could be argued that since a remaining normal ovary undergoes compensatory hypertrophy after unilateral spaying because of increased available gonadotropin the operation ought also to promote the survival of the grafts. An experiment to test this suggestion (150) failed to show any effect of the unilateral spaying

Next is there any evidence that injected extra gonadotropin can increase the survival of grafts? Ferguson and Kirschbaum (1954) (150) studied the effect of giving pregnant mare serum on the survival of ovaries grafted from C57BL mice to castrated Strong A mice. In control experiments the grafts had usually degenerated within three weeks. With treatment the grafts contained viable tissue for up to five weeks but they degenerated despite the continuation of treatment.

Ingram and Krohn (1956) (159) found that treatment with gonadotropin (PMS) increased the proportion of rat grafts which survived

at 21 days but they did not believe that the graft would have survived permanently. Estrogen also promoted the survival of the grafts, a fact which they ascribed to the release of hormone by the pituitary. Deanesly (1956) (32) found that injections of PMS had no effect on the amount of lymphocytic infiltration around homografts, although Harris and Eakin (1949) (44) believed that the extent of infiltration was less in spayed hosts than when the ovaries were intact.

It has been mentioned above that a graft remains in a state of "suspended animation" if even part of the host's own ovarian tissue is allowed to remain in its natural position. But what happens if additional ovarian tissue is transplanted? Can the ovarian-hypophyseal balance be upset by the addition of several ovaries and in what way? Experiments of this sort are unfortunately subject to doubts as to the genetic make-up of the grafted tissue.

According to del Castillo and Calatroni (1930) (165) a third ovary was transplanted to the kidney of adult normal female rats without affecting the vaginal smears or estrous cycles. In the successful grafts (41 per cent) there were no corpora lutea and only a few follicles. Takewaki (1933) (43) made similar observations on estrous cycles but noted that corpora lutea developed. Neither of these workers agrees with the results of Friedman and Nice (1930) (166) who found that the estrous cycles were more irregular and more frequent than normal when two extra ovaries were transplanted.

Brewster and Zuckerman (1949) (167) also found that the estrous cycles of rats were unaltered by the addition of either two or six ovaries subcutaneously. They did find, however that the size of the host's ovaries decreased and that the number of oöcytes in the normal ovaries was less than usual even when the number remaining in the graft was taken into account. They felt that there might be some organ-specific immunologic reaction which affected the host's ovaries as well as the graft. Further work by Mandl and Zuckerman (1951) (168) however failed to confirm these changes which were ascribed to deficiencies in the design of the original experiment.

No experiments have yet been reported in which multiple isografts of proved acceptability to the host have been made. Until this is done the capacity of the pituitary to accommodate its secretions to the requirements of excess ovarian tissue as it does to decreased amounts will remain uncertain. What these experiments certainly show is that Halsted's law is no more to be taken as absolute dogma for ovarian grafts than for grafts of the other endocrine organs.

Apart from the possible effect of ovariectomy which has just been considered, more specific methods of attacking the immunity producing system have been tried at various times.

As early as 1915 Mitchell (169) tried to modify the response by injections of peptone but they seemed to intensify rather than diminish the reaction. Arnold (1928) (170) and Miller and Lampton (1941) (171) tried to "blockade" the reticuloendothelial system with trypan blue but without much success. More recently Ingram and Krohn (1956) (159) increased the proportion of homografts in rats which were surviving 21 days after operation by giving cortisone but there was no suggestion that survival would have been permanent or even greatly prolonged.

Parkes (1957) (172) applied the technique developed by Kaliss for modifying the response to grafts of tumors by pretreating future hosts with an intraperitoneal injection of an ovarian homogenate. A higher proportion of treated rats tolerated the subsequent ovarian graft but the method was not successful when applied to mice. An attempt has been made by Ten Berge and The Tik Lok (1953) (173) to reproduce the advantages which accrue to a graft when it is divorced from the host's blood supply by covering the ovarian homografts with amnion. Nine rabbits received such grafts and viable cells were identified in the grafts up to seven months later.

Finally, to revert to Parkes and Billingham's work, it still seems that though ovarian grafts are not absolutely privileged, a genetic difference sufficient to cause the death of a skin graft will not necessarily kill an ovarian graft. Possible explanations for this apparent privilege are very numerous.

1) The amount of tissue grafted is too small to elicit a full reaction especially since much is destroyed soon after grafting. But evidently the amount of tissue is often entirely adequate to elicit a complete and rapid reaction.

2) An actively growing endocrine graft can inactivate the antibodies produced by the host. The only evidence here and that indirect is that on the contrary grafts of vaginal tissue

survive no longer than usual if they are stimulated to active growth by injections of estrogen (Krohn 1955) (174) If the antigens responsible for eliciting an immune response are nucleoproteins derived from nuclear activity one might indeed expect increased cellular activity to accelerate the response. On the other hand, the typically dormant small follicles in the ovary might be expected to remain unaffected.

3) As Ingram and Krohn (1956) (159) point out, the conditions of grafting, as between skin graft and ovarian graft, are very different. The one is orthotopic to a richly vascularized bed the other is subcutaneous to less vascular fatty connective tissue with probably less efficient lymphatic drainage.

4) The histocompatibility antigens produced by ovarian tissues are fewer or less capable of stimulating an immunity response than those from skin

5) The graft survives because the hormonal imbalance caused by the stress of operation and the removal of the gonads increases the output of adrenal corticosteroids, which are known to increase the proportion of surviving homografts.

If the observations which seem to require explanation are indeed correct, there is nothing at present to decide between the alternative explanations.

### Effects of Grafting the Ovary

#### *Grafting of Ovary and Onset of Puberty and Menopause*

It has already been mentioned that the maturation of young ovaries is accelerated by transplantation into an adult environment (7 8) Long and Evans (1922) (41) grafted 22- to 29-day-old rat ovaries into adult rats and found that estrus reappeared again 6 to 8 days after the operation. In three out of their seven animals, a second estrous period followed 4 0 and 10 days later After this there were no further estrous cycles and the transplants became atrophic It must be assumed that the young grafts had been destroyed by a homograft reaction which went unrecognized by Long and Evans. We now know that such young grafts if compatible, may retain activity for long periods of time, and indeed as long as the natural life of the host. Long and Evans also attempted the reciprocal experiment by transplanting ovaries from adult to young animal The experiment failed however and there was no hastening of the opening of the vagina or of estrous vaginal smears. Long and Evans mention that these negative results might be related to what they term protein incompatibility '

Foà s observations (7 8) were confirmed by Lipschütz (1925) (175) who also carried out the converse experiment and found that the transplantation of mature ovaries into a prepubertal host does not induce precocious feminization of the host (176 177)

May (1940) (46) who transplanted newborn ovaries to the anterior chamber of the eye of pubertal spayed rats, reports that in 4 weeks lutein bodies like those of a one-year-old rat may develop while Dunham Watts and Adair (1941) (178) found that ovaries of a newborn rat also transplanted to the eye of spayed adult hosts brought on estrus in 9 to 26 days (compared with the 13 to 17 days reported by May the average of about 8 days being usual in subcutaneous adult grafts, and the 7 to 14 days for adult grafts to the anterior chamber of the eye) Regular cycles followed for more than 12 months. Similar experiments have been reported by Engle (1929) (179), Goodman (1934) (180) and Pfeiffer (1934) (181)

There is some evidence, however that the act of transplantation may itself influence the age of onset of puberty Thus Greep and Jones (1950) (182) as had Hohlweg and Dohrn (1932) (183) and Mandel (1933) (184) found that autotransplantation of 25-day-old ovaries was followed by early opening of the vagina in rats. They suggested that during the interval between grafting and revascularization the animal could be considered to be the equivalent of a spayed animal. Removal of an ovarian check on the pituitary even at this early age, allowed the gonadotropic activity of the pituitary to increase As soon as vascular channels were reestablished the graft responded to the gonadotropin and the production of hormones by the graft was accelerated. Hohlweg (1952) (185) agrees that a single precocious estrus occurs but adds that normal cycles do not recur until the normal time at which they would be expected. Mandl and Zuckerman (1951) (186) repeated these experiments by Greep and Jones but failed to confirm them. A second series of experiments suggested to them that the nonspecific stress of operation or of anesthesia might be sufficient to stimulate early activity in the pituitary In Almquist and

Jacobsohn's experiments (1956) (187) (using intraocular as opposed to subcutaneous autografts in young rats) the acceleration of the vaginal opening was not statistically significant. Anesthesia or control implants of uterus were ineffective. If very young (<24-hour-old) donors were used, the interval before the vagina opened was prolonged, i.e. such young ovaries are temporarily refractory to any stimulus.

It seems clear therefore that after a short period of almost absolute insensitivity the young ovary is capable of responding to gonadotropin, though its sensitivity may still be subnormal. Since the pituitary of a young animal is known to contain gonadotropin it must be presumed that the onset of puberty depends on some factor which releases preformed gonadotropin from the pituitary.

Harris and Jacobsohn (1952) (188) grafted young pituitary tissue under the median eminence of hypophysectomized adult rats, and showed that estrous cycles returned within 8 to 35 days. This finding indicated that the immature pituitary as well as the immature ovary is capable of normal cyclical behavior at an early age and perhaps means that the prime factor determining the onset of puberty lies in the hypothalamus or some other part of the brain which is able to stimulate the anterior lobe of the pituitary. Such a notion would fit in with the rare but well attested cases of hypothalamic tumor which result in a premature puberty in children.

It is generally believed that when reproductive activity fades or ends as an animal becomes older it is primarily because the ovary has failed. Frequent attempts to rejuvenate aging females by transplanting young ovarian tissue have been based on this general belief (see especially Pettinari, 1928 (31)) but have met with little success. Yet the technique of transplantation can contribute much to the study of the aging of the ovary and of the other endocrine organs. The whole problem has been discussed at length by Krohn (1955) (180).

In Lifimov's (1951) (190) experiment young ovaries continued to secrete hormones after a prolonged sojourn in old hosts whose natural estrous cycles had come to an end. Pituitary function is therefore thought not to be affected or may even be increased. Takewaki (1933) (191) points out that ovarian grafts which have been active for up to a year become gradually more and more inactive. The replacement of such inactive grafts by young grafts is followed by the return of estrous cycles in the host. This result would be further evidence for the adequacy of the pituitary and the failure of the ovary in old age. However Takewaki also reports that such inactive grafts can still induce estrus when they themselves are transplanted into young spayed animals. The inactivity therefore of an old graft is not entirely due to its own inadequacies. It must be admitted that any explanations for the timing of either the beginning or the end of reproductive life rest on most inadequate concepts of interrelations between the levels of hormone production and of sensitivity to trophic hormones.

### *Sex Differences in Pituitary Function Disclosed by Ovarian Grafts*

Sex differences in the production of gonadotropins by the pituitary gland can readily be demonstrated by means of ovarian grafts. Thus it is clear that ovaries which are transplanted into castrated males will show active follicular development only; the follicles do not ovulate and corpora lutea are not formed (128, 180). If a piece of vaginal epithelium is grafted together with the ovary into a male, the epithelium remains in a state of persistent cornification. Gonadotropin is apparently secreted at a constant rate in the male and does not fluctuate cyclically as it does in the female. Since the follicles of ovaries grafted to the male respond to injected ovulating and luteinizing hormones, it is inferred that the male pituitary secretes only follicle stimulating hormone (FSH) unlike the female pituitary which secretes luteinizing hormone (LH) as well. Takewaki (1933 and 1942) (192, 193) however believes the lack of LH is not absolute and that the male pituitary produces a little but not much, since unlike other workers he has found some luteal tissue in ovarian grafts transplanted into castrated males. In any case it is hard to see how the interstitial cells in the testis can be maintained without the pituitary producing LH or something like it.

Work carried out by Pfeiffer (1936 and 1937) (93, 194) was designed to investigate these sexual differences between the hypophyses of males and females. He found that the pituitary at birth is not yet sexually differentiated; in the female it can be developed towards the male type of function by transplant of the testis. Such transplants apparently suppress the capacity

to produce luteinizing hormone, which is characteristic of the female. If the pituitary of a newborn female is masculinized in this way then the ovary subsequently does not ovulate and the vagina remains in constant cornification.

Harris and Jacobsohn (1952) (188) however found that the anterior pituitaries of adult male rats would restore normal estrous cycles and permit pregnancy to occur in adult hypophysectomized female rats provided that the grafts were revascularized by the pituitary portal vessels. Clearly therefore, Pfeiffer's explanation of the mechanism by which the pituitary becomes sexually differentiated is oversimplified. Harris and Jacobsohn feel that the differentiation in either the male or female direction, thought by Pfeiffer to occur normally before puberty is not absolute and is not inherent in the pituitary tissue itself but depends on influences deriving from the hypothalamic connections to the pituitary via the hypophyseoportal system. Martinez and Bittner (1956) (195) have also found that not only male but also female pituitaries grafted to castrated male mice which have ovarian and vaginal grafts, induce constant and not cyclical activity The internal metronome which regulates the rhythms of reproductive processes seems, therefore, to lie outside the pituitary itself

*Effect of Ovarian Transplants on Susceptibility of Mice to Cancer*

Strains of mice vary enormously in their susceptibility to cancer particularly to mammary cancer The proportion of animals dying from the latter disease may range almost from 0 to 100 per cent. In the A strain of mice there is the added peculiarity that the susceptibility is high in breeding mice and low (almost nil) in virgins. The incidence of mammary cancer in these strains can be modified by the administration of sex hormones or by spaying. It is therefore clear that transplantation of ovarian tissue would be expected to provide valuable information about the control of susceptibility to this form of cancer

Thus transplants into virgins of the ovaries of mice that have had litters would help to reveal whether there were any particular structures associated with breeding that are responsible for the increased incidence. If an exchange of ovaries did not alter the cancer rate the increase might be related to the amount of suckling and mammary gland activity in breeding animals, and not to the pattern of hormones secreted by the ovary

Early work on this theme was unsuccessful (196) primarily because the strains of mice used were insufficiently inbred, but later work (197, 198) has amply confirmed observations that mammary tumors can be induced by ovarian transplants.

Ovarian grafts will also stimulate the development of mammary gland rudiments (199) in male mice which will develop cancer in 40 per cent of experiments (158) Since this figure is well over the 4 per cent found in normal virgins it indicates not only the carcinogenicity of some ovarian secretion but also the operation of another factor in the male which results in the graft receiving more of a stimulus than it is normally subjected to in the female. This factor may be the constant pituitary activity which is thought to differentiate male function from the characteristically cyclic behavior of the female.

The difference in the incidence of cancer between virgin and breeding A strain mice has already been mentioned. The likelihood of a tumor increases with the number of litters, or with the frequency of pseudopregnancy (if mating with vasectomized males is allowed) Changes in the incidence of cancer must be related therefore, to hormonal changes in pregnancy and Silberberg and Silberberg (1949 1950) (200 201) have shown that the additional stimulus of several grafted pituitaries will increase the chances of a tumor developing The combined effects of ovarian and hypophyseal grafts seem to be greater than those of ovarian tissue only but pituitary grafts are ineffective unless the ovaries are present (199)

Further observations by Huseby and Bittner (1948) (202) have been concerned with the effect of transplanting C3H and A strain ovaries into spayed hybrid (C3H × A) mice, in order to study the effect of the environment on the susceptibility to cancer Judged by vaginal smears the output of hormones by grafted A C3H and A × C3H ovaries in the common environment were indistinguishable for 10 months after grafting. Tumors developed in all three combinations, but hybrids carrying A strain ovaries developed their tumors later However 57 per cent of them finally developed tumors, compared with 4 per cent in ordinary virgin females. This fact indicates as did Huseby and Bittner's (1951) (158)

finding referred to above that the actual process of grafting increases the susceptibility of the ovary to undergo changes which lead to malignancies elsewhere. Hummel, Fekete and Little (1953) (54) have also found that transplantation increases the likelihood of a cancer developing in the grafted ovary itself

It is interesting to note that morphologic differences which are ordinarily evident between C3H and A strains, disappear after a period in the hybrid host. This observation shows clearly the value of transplantation as a method for dissociating the effects of genetic make-up from those of the environment

## TESTIS

The special place that the testis holds in man's emotions must be held responsible for the repeated attempts to restore sexual activity and virility by means of grafts and to relieve the degenerative changes of old age which were thought to follow testicular atrophy The literature is indeed full of optimistic clinical reports of successful transplantation *e.g* Lespinasse (1913) (203) Lydston (1916) (204) and Thorek, (1924) (205) to be contrasted with a series of experimental investigations, which show complete failure to obtain successful grafts, at any

TABLE 9

*Proportions of successful grafts of testis in rats (from Turner (1938) (216))*

| Site of Transplant | Transplants Recovered | |
|---|---|---|
| | *Proportion* | *%* |
| Intraperitoneal | 6/17 | 35 3 |
| Intramuscular | 7/8 | 87 5 |
| Subcutaneous | 12/22 | 54 5 |
| Liver | 7/13 | 53 0 |
| Kidney | 3/6 | 50 0 |
| Scrotum | 3/8 | 37 5 |
| Total | 38/74 | 51 3 |
| Intraocular | | |
| Normal males | 60/74 | 80 2 |
| Castrated males | 40/41 | 00 0 |
| Normal females | 4 /52 | 90 4 |
| Spayed females | 20/22 | 90 9 |
| Pregnant and lactating females | 20/20 | 100 |
| Total | 193/ [illegible] | 91 0 |

rate in mammals. Such efforts culminated in the experiments of Voronoff (summarized in 1923) (206) who encouraged the illusory hope that heterografts from ape to man would be successful, but whose work is now discredited. Not only do the grafts fail but the amount of hormone originally present in the graft is insufficient to have even a temporary effect (207 208) Most of this and subsequent work is summarized by Hoskins, (1925) (209) Rollet, (1927) (210) and Moore (1926 1930 and 1931) (211–213) and very little that is new has been reported since that time.

A few general conclusions can be summarized. First most workers agree that isotransplant are successful whichever sex the host may be Neither the site for transplantation nor the presence or absence of the gonads has much influence on the survival of the graft. But while the production of male hormone continues with little if any diminution for as long as is normal (for example up to 8 years in a cat (214) spermatogenesis takes place only if the graft is made into the scrotum or ear (211) The ambient temperature of the anterior chamber of the eye also provides a suitable environment (215 216)

It is generally agreed too, that the success or failure of a graft depends on the genetic relationship between host and donor but the information bearing directly on this point is slight. Blockade of the reticuloendothelial system with colloidal iron or trypan blue is said to improve the chances of a homograft surviving (217)

Turner (1938) (216) carried out the most extensive series of experiments so far reported. He used young donors and young mature hosts from a closed colony of Wistar rats. His results are summarized in table 9 From these figures it is apparent that about 50 per cent of grafts were recovered from sites other than the eye Spermatogenesis was found only in intrascrotal grafts. Though transplants of prostatic tissue made at the same time had been stimulated, the normal appearance of the pituitary was not always maintained and some of the characteristic castration cells which depend on a deficiency in androgen production were seen It is also evident from the figures that grafts of the testis will survive even if there is no lack of testis hormones. In other words Halsted's law cannot be rigidly applied.

Unlike young ovarian follicles, young male germ cells showed no signs of precocious maturity in an older environment, nor could early maturation be induced by injections of extracts or implants of pituitary which did, however stimulate the production of androgen. The grafts developed sperm less readily if the hosts were female, probably because the female pituitary has a smaller gonad stimulating potency than the male.

Browman's (1937) (218) results with both mice and rats are similar to those of Turner In addition he found that heterotransplants from mice to rats or *vice versa* were always unsuccessful and that failure was uninfluenced by a variety of treatments such as irradiation splenectomy, adrenalectomy, injections of tissue extracts or India ink, which might possibly have modified the reaction. He adds the findings that heterotransplants from mice to rats survive for up to four days in the rat and become active when restored to the original mouse donor

Pfeiffer's observations on the effect of testicular grafts on pituitary function in newborn mice are described on p 420

Williams (1949 1950) (219 220) has studied microscopically the behavior of autografts of the rabbit testis in transparent ear chambers, where transplants survived for up to 14 months. The pattern of blood vessels in the graft tissue varied according to the presence or absence of interstitial tissue cells. Williams suggests that this may indicate the secretion of a spreading factor by the interstitial cells.

Such chambers allow repeated observations to be made on the components of the graft. The interstitial cells appear to develop from fibroblasts, acquire cytoplasmic granules, and finally regress over a period of about nine months to become indistinguishable again from the surrounding connective tissue. Williams noted that the condition of the tubules seemed to depend on the fate of the interstitial cells. If they were present, Sertoli cells persisted and formed secretions. If they were absent, the tubules closed became fibrosed and resembled the tubules in old age. The importance of this observation lies in the suggestion that tubular fibrosis, which is such a common feature of the old testis, can be reproduced in young animals. It may therefore, be related either to closure of the tubules or to some inadequacy of the interstitial cells, rather than a primary concomitant of old age.

As might be expected, experiments on intrasplenic grafts—so fruitful in the case of the ovary—have also been carried out on the testis. Kricheaky Benjamin and Slater (1943) (221) found that testis autografts to the spleen of castrated rabbits were without effect on the atrophic prostate unless vascular adhesions occurred which had the effect of allowing the blood draining from the graft to bypass the liver Li, Pfeiffer, and Gardner (1947) (222) also found in A strain mice that the liver totally inactivated the androgen that is produced by such a graft, which resembles a cryptorchid testis. So far, then the testis behaves like the ovary But Li and coworkers were unable to induce tumors in the grafts although A strain mice readily develop tumors in normal scrotal testes under some conditions of hormonal imbalance *e.g*, if estrogen is administered. Biskind and Biskind (1945) (223) agree that the liver destroys androgen in the rat and were able to report a tumor in one intrasplenic graft which resembled a mixed granulosa and thecal cell tumor of the ovary

The testis, like the ovary can be preserved by freezing to −79° or −190°C in a glycerol/saline medium (224) The endocrine function of the thawed tissue was tested by studying the effects of the grafts on sexual behavior and on the weight of seminal vesicles in castrated adult males. Thawed 7 to 9-day-old testes will later show full spermatogenesis if transplanted to the scrotum. Development is not so complete in subcutaneous grafts. A detailed histologic report of the findings is given by Deanesly (1954) (225)

## PITUITARY

Nowadays it is platitudinous and almost old fashioned to say that the anterior lobe of the pituitary is the conductor of the endocrine orchestra but no matter how autocratic the conductor is he is still subject to many restraints. There is excellent evidence that the day to-day control of the activity of the thyroid adrenals and gonads depends on an interaction between the levels of circulating hormones from these glands and the pituitary trophic hormones. By a familiar "push-me-pull-you" mechanism an increase in say the output of thyroid hormone is compensated for by a fall in the pituitary's release of

thyrotropin and *vice versa*. But this usually very effective control mechanism is not all. The pituitary is also under a mysterious control exercised by a Svengali-like central nervous system. There is equally good evidence that the gland responds to changes in the external environment which can be mediated only through the central nervous system. To take just one example we know that increasing the amount of light incident on the eye in the ferret (or decreasing the amount in sheep) sets up a train of events whereby a stimulus of some sort travels *via* the optic nerve to the brain. Here the pathway is lost until, finally, the anterior lobe of the pituitary is triggered off to secrete gonadotropin in what would ordinarily be an anestrous period of the year. The pituitary's responses to other changes in the outside environment, such as its control of thyroid function following temperature changes, or its production of ACTH to meet a wide range of so-called stresses, also spring to mind as examples of the close links between the central nervous system and the pituitary.

The only direct anatomic connections between the hypothalamus and the pituitary are the nerve fibers and blood vessels in the stalk. Many but not all workers believe that an insignificant number of fibers reach the anterior lobe so that we are left, according to Harris, with the pituitary portal system of blood vessels as the only pathway whereby as yet unknown chemical messengers can transmit information and instructions from the brain to the pituitary. A detailed discussion of the pros and cons of the vigorous controversy about the role of the portal vessels in the control of pituitary function is out of place here and can be found elsewhere (220–225) but it is obvious that a crucial method for studying the problem is to remove the pituitary from its normal position in the sella turcica and to transplant it elsewhere. For if normal function can be achieved after transplantation there can be no specific control of pituitary function *via* the stalk or hypophyseal-portal vessels. Most recent experiments on transplanting the pituitary have therefore been directed to these ends and have not been concerned primarily with the problem of the homograft reaction or of Halsted's law as they have with other endocrine glands, though the results of some have been vitiated by overlooking the complications introduced by the use of homografts and others present positive evidence bearing on both points. The very meager reports on the results of transplanting either the pars intermedia or pars posterior do not justify a special section and will be mentioned wherever they are appropriate.

The experiments will now be reviewed. First those which are concerned with evidence for the general capacity of pituitary grafts to maintain normal growth, development, and reproduction, then those dealing with the graft's capacity to control specific endocrine organs, and finally with its responses to various hormonal factors.

Crowe, Cushing and Homans (1909) (229) seem to have been the first to make such studies at a time when it was erroneously believed that total hypophysectomy was rapidly fatal. The best that they could report was that the early fatal consequence of total hypophysectomy in dogs could be postponed by the immediate autotransplantation of the gland into the cortex of the brain. In three out of thirteen experiments they found areas of viable anterior lobe cells about 25 days after operation.

Further attempts to transplant the pituitary do not seem to have been made until the 1930s and reports are concerned with the morphologic characteristics of the grafts only. Function could not be assessed because the hosts were not hypophysectomized. Gardner and Hill (1931) (230), using inbred strains of mice, transplanted the pituitary from littermates into the testis. The grafts were recovered 3 to 12 months later. They were well vascularized and seemed to have the normal arrangement and proportion of cell types.

In Hill and Gardner's experiments on mice (1936) (231) the grafts were able to maintain testicular development or to restore the atrophied testes of the hypophysectomized animal. A female pituitary grafted into a male mouse was also capable of supporting the growth and function of an ovary which was grafted at the same time. Hill and Gardner refer to work published by Strong (1936) (152)—virtually the only report of its kind—which showed that genetic uniformity of host and donor was necessary for the success of intratesticular grafts of pituitary tissue.

May (1935) (232) transplanted pituitary tissue from newborn rats into the anterior chamber of male rats which had been hypophysectomized 2 months earlier and which showed by their lack of growth that they were completely hypophysectomized. In two experiments the rats began to grow again, though not to the extent

of their normal controls. The testes descended into the scrotum and spermatozoa were formed. In a further paper May (1937) (233) reports similar experiments on hypophysectomized female rats. Within a few days of grafting the rats became more active and they began to put on weight. One rat had normal estrous cycles, became pregnant and gave birth to a litter. The eye which contained the graft was then removed and the hypopituitary state returned. May believes that the pituitaries of newborn animals have to become mature before they can have an effect on the sexual organs of the host.

Haterius, Schweizer and Charipper (1935) (234) transplanted half pituitaries into the anterior eye chamber of unrelated rabbits or guinea pigs. At intervals of 16, 25, and 31 days after implantation the grafts showed normal-staining cells and mitoses. There was some lymphocytic infiltration, especially around the edges of the grafts. Grafts were recovered from guinea pigs at intervals of 36, 91 and 114 days after grafting. There was definite lymphocytic infiltration of the grafts at 36 days but even at 114 days individual cells were well defined. The grafts did not appear to become vascularized, and presumably obtained their metabolic requirements from the fluid of the anterior chamber in circumstances which may have minimized any homograft reaction.

Martins (1936) (235) grafted anterior pituitary tissue into either the eye or the kidney of hypophysectomized or castrated rats. Growth never returned to normal, and ovarian development was retarded. The thyroid and adrenals were also atrophied. Such pituitary tissue as remained in the grafts was not properly differentiated.

Haymaker and Anderson (1936) (236) attempted to modify the antigenicity of pituitary tissue by culturing it before transplantation into hypophysectomized rats. The amount of tissue which they grafted only represented a very small proportion of the normal weight of the gland. In a few instances they were able to report histologic repair of the host's endocrine organs, and very frequently increase in body weight. Despite this apparent evidence of success they never found any of the grafted pituitary tissue at autopsy. It is therefore difficult to agree with their conclusion that the results were due to the effects of the grafts.

Buxton (1936) (237) also transplanted the pituitary into the anterior chamber of the eye of rats. In most of his experiments autotransplants were obtained by simultaneous hypophysectomy but he also used some homotransplants. Seven out of the forty-two animals he prepared showed viable vascularized transplants at the end of three to six weeks but the total number of his successes was too small to judge the relative viability of autografts and homografts.

Phelps, Ellison, and Burch (1939) (238) grafted pituitaries intramuscularly in hypophysectomized rats of an inbred strain. They describe the peripheral survival and central necrosis that are characteristic of endocrine grafts: normal appearances had been restored 20 days after grafting. Histologically the grafts seemed adequate and they responded normally to the administration of estrogen but restitution of the changes brought about by hypophysectomy was only very slight.

Wolfe, Kirtz, and Loeb (1940) (239) reported that multiple transplants of anterior pituitary tissue from inbred strains of mice live for at least 7 to 10 months after transplantation subcutaneously. Most of the surviving tissue was made up of chromophobe cells but there were usually a few eosinophils as well. Basophils were not seen but it must be remembered that they are very difficult to see in the normal pituitary of a mouse. The transplants were able to stimulate the host's ovaries and to cause changes in the mammary glands.

Halsted's law cannot easily be applied to the circumstances of pituitary transplantation, for there is no known secretion (unless it be a hypothalamic one) of which the pituitary stands in need. But the evidence for the success of grafts of supplementary tissue in intact animals adds further evidence against the general concept.

Westman and Jacobsohn (1940) (240) homografted tissue from newborn or young rats into the anterior chamber of hypophysectomized females. The grafts were well vascularized and the mass of the graft was either as large as, or larger than the size of a normal anterior lobe. Despite histologic evidence of normality the transplants apparently had no gonadotropic activity. There were no estrous cycles and the ovaries were completely atrophied. On the other hand, some growth was observed and the adrenal glands underwent only partial atrophy.

Schweizer, Charipper and Haterius (1937) (241) report further work using transplants to the anterior chamber of the eye of hypophy-

sectomized adult female guinea pigs. The tissue transplanted was obtained from another freshly killed female whose genetic relationship is not described. When the guinea pigs were killed 3 to 13 weeks later the transplants showed reasonably normal structure and a definite predominance of basophilic elements (usually thought to be concerned with the formation of gonadotropin). Eosinophils were rare, and there were only occasional chromophobe cells. About two months after grafting the animals showed constant vaginal cornification. The ovarian follicles were well developed but corpora lutea were absent. The uterus and the mammary glands were hypertrophied. In addition to these signs of ovarian activity the thyroids and adrenals were also well maintained. Schweizer and colleagues (1937) (241) conclude, therefore, that the grafts, divorced from their normal situation, were able to produce normal amounts of FSH only but could not secrete LH. They say: "It is not inconceivable moreover that implantation on the original site of the gland would favor the reestablishment of normal neurovascular relations which would essentially defeat the purpose of transplantation. There is, however, no proof that such is the case." This remark is a very clear forerunner of the work carried out later by Harris and Jacobsohn (1952) (188). Schweizer, Charipper and Kleinberg (1940) (242) extended their observations to adult male hypophysectomized guinea pigs. Grafts from both sexes were used and gave identical results. The reproductive tracts of the hosts, which were killed from 50 to 160 days later, were well maintained. All stages of spermatogenesis could be found in the testes and the epididymes were well filled with sperm. The interstitial tissue of the testes was not so abundant as in normal animals—a finding which is attributed to a low level of secretion of LH. These two reports reveal a difference between the amounts of LH secreted by a pituitary depending on whether it is transplanted into male or a female. Schweizer and associates (1940) (242) somewhat inadequately explain the difficulty by proposing that while the amount secreted is the same in both sexes, it is sufficient only to stimulate the testis. Its effects on the ovary are not visible because the ovary has a higher threshold. In contrast to earlier work on females, the atrophy of the adrenal was not affected by the transplant.

Goldberg, Knobil and Greep (1955) (243) implanted fetal pituitaries into the anterior chambers of hypophysectomized male rats where they remained for 2 to 8 months. In most instances they found not only anterior and intermediate lobe tissue but also tissue from the posterior lobe. The anterior lobe cells were mainly agranular chromophobes with a few well granulated chromophils. Evidence for the function of the transplants varied according to the endocrine target organ chosen and could in no case be related to the histologic appearance of the graft. Thus the body weights were greater than those of the hypophysectomized controls, so too were the adrenal weights and the response to unilateral adrenalectomy. On the other hand, the weights of the thyroids, their histologic appearance, and the lack of response to treatment with propylthiouracil suggested that no thyrotropin was being produced. In 41 per cent of the rats, spermatogenesis and ability to fertilize normal females were noted, but even so the amount of androgen secreted was diminished.

Further work on mice has been carried out by Mühlbock and Boot (1956) (52). Transplants which contained only chromophobes appeared to improve the viability of hypophysectomized hosts and increased the weight of the adrenals but showed no sign of producing either FSH or LH. Transplants into normal mice induced pseudopregnancy and had effects on mammary tissues similar to those of injected prolactin. Courrier (1956) (244) transplanted the pituitary to the juxtathyroid position in hypophysectomized rats. Thirty and 54 days later the testes showed full spermatogenesis though the adrenals and thyroid were both inactive. The grafts were well vascularized but the cells were dedifferentiated.

Anterior pituitary tissue has been grafted into the anterior chamber of the eye in ferrets by Thomson (1957) (245). Many grafts succumbed to the homograft reaction but occasionally some have shown satisfactory cellular differentiation (fig. 184).

The morphology and histochemistry of the pituitaries of newborn mice which had been isotransplanted to the anterior chamber of the eye have been examined in detail by Siperstein and Greer (1956) (210). The observations were carried out between one day and 11 months after grafting. Much of the center of the graft was necrotic during the first 8 days, the nuclei of



FIG 184 (*left*) An intraocular pituitary homograft (ferret) Section kindly lent by Dr A P D Thomson Healthy looking tissue made up of well differentiated acidophils (dark-staining) and chromophobes No basophils The vessels have been perfused with India ink ×106

FIG 185 (*right*) A subcutaneous pituitary isograft in a normal mouse Large mass of healthy looking but degranulated anterior lobe cells Distinct and possibly hypertrophied zone of pars intermedia cells ×24

cells could no longer be seen, though the cytoplasm still stained specifically Revascularization was well established by the eighth day At 11 days the normal structure and cell outlines had been restored apart from some collections of debris mitoses had become frequent. Chromophils were far fewer than normal at all times and the grafts were made up mainly of two types of chromophobes. At 14 months the grafts were enlarged and full of large chromophobes containing a very hypertrophied Golgi image The chromophobes contained ample RNA in the cytoplasm and were presumably active metabolically They somewhat resembled the chromophobes in the pituitary of an animal treated with estrogen.



FIG 186 High power view of part of figure 185 The active pars intermedia is shown together with the degranulated anterior lobe cells ×130

After an early period of regression the pars intermedia hypertrophied or at least recovered its normal size (figs. 185 186) Hypertrophy of the pars intermedia has also been reported after stalk section or hypothalamic lesions. The experiments have been used to support the idea that separation of the tissue from the brain removes some growth inhibiting factor which might come from the hypothalamus. But the most recent quantitative studies have not confirmed the facts on which these suggestions were based in the rabbit (247) The posterior lobe was recognizable during the first 8 days but by 15 days it had disappeared.

Other recent work has been concerned with the local effects of pituitary transplants which have been placed in the testis. Petrovic Weill, and Deminatti (1953) (248) and Petrovic, Deminatti and Weill (1954) (249) implanted small fragments of the anterior pituitary into the testes of immature and adult guinea pigs, where they stimulated local development of the interstitial tissue near the grafts but retarded the process of spermatogenesis. The grafts are said to have retained a normal histologic structure though they were apparently homografts. This histologic finding is confirmed by Aron and associates (1953) (250) who ascribed the absence of any homograft reaction to a protective action of the testis. May (1955) (251) removed the very atrophied right testis from young mice one month after hypophysectomy and grafted the pituitary of a newborn mouse to the tunica albuginea of the

other testis A month later the transplanted pituitary was found to have grown and to contain typical anterior pituitary cell The tubules of the testis were enlarged and there were numerous normal spermatozoa. Aron and associates (1956) (252) have carried their earlier work further by transplanting parts of the pituitary which they knew to be made up mainly of acidophils or of basophils Either type of graft seems to have the same effect locally on the testis and they doubt, therefore, whether the distinction between alpha and beta cells as the producers of FSH and LH respectively is tenable

**Orthotopic Transplantation of Pituitary**

Two papers on orthotopic transplantation may now be described which serve as an introduction to the more extensive work by Harris and Jacobsohn. The first is that of Greep (1936) (253) who transferred either auto- or homografts from a highly inbred strain into the empty sella turcica of 28-day-old hypophysectomized rats. The animals grew rather more slowly than normal but underwent normal sexual development and some became pregnant. In this sort of experiment it is impossible to be certain that the entire pituitary was removed but even so the results seem to indicate that the grafts were active. Female rats carrying a single newborn male pituitary never became sexually mature, but five such pituitaries per host were effective. Male hosts showed normal reproductive behavior whether they received male or female pituitaries Cutuly (1941) (254) made successful autotransplants into the anterior chamber of *the eye of six male rats and into the sella turcica* of four male rats The grafts into the eye were poorly vascularized and were composed mainly of chromophobes with only occasional basophils and eosinophils Tissue transplanted into the sella turcica however was better vascularized and appeared more normal histologically It contained numerous fairly large eosinophils and many small basophils Cutuly found that the histologic character of the graft was not necessarily a satisfactory index of functional activity for grafted tissue in the eye of an animal which showed all the signs of hypophysectomy might be indistinguishable from that seen in the eye of a rat whose transplant was apparently maintaining normal function Transplants into the sella stimulated the growth of the adrenals but transplants into the eye did not. Neither type of graft affected the thyroid

Cutuly s conclusions may be quoted It seems clear that autoplastic grafts of the pituitary gland severed from their normal neural communications were capable of maintaining the entire reproductive tract of hypophysectomized male rats in a condition anatomically and functionally indistinguishable from the normal." "Anterior lobe tissue grafted into the eye was able to function as well gonadotropically as sella grafts and indeed as well as an intact pituitary gland. This would appear to eliminate participation of the nervous system in the release of the gonadotropin responsible for complete testicular activity

Harris and Jacobsohn (1952) (188) undertook further experiments designed to support their thesis that the anterior pituitary is under a neural control from the hypothalamus which is mediated by the transmission of controlling substances *via* the hypophysioportal vessels and that transplants fail to function when they are unable to receive these materials directly through the portal vessels. Using hypophysectomized rats of an inbred strain they first transplanted pituitaries from the female recipient s own offspring, either under the median eminence or into the temporal lobe a few millimeters laterally or into the emptied pituitary fossa. Similar experiments were also made with adult donor material and with male recipient

Estrous cycles returned *in all twelve* completely hypophysectomized females which received their offsprings pituitaries under the median eminence Six of them became pregnant *but were unable to rear their young* owing to the absence of a suckling reflex. Milk secretion could be restored by injecting oxytocin The ovaries adrenal and thyroid glands were normal. The grafts were richly vascularized and well differentiated (including the pars intermedia) and acidophil, basophil and chromophobe cells were all seen.

Female rats which received adult grafts under the median eminence did not give such good results. Only *four* of the ten had estrous cycles and only two became pregnant. The grafts were well vascularized and they were infiltrated with lymphocytes and fibrous tissue The grafts in adult males although vascularized from the portal system were also infiltrated with lymphocytes

In contrast to the findings in the first group of experiments none of the ten animals which received grafts into the temporal lobe came into estrus and all the endocrine organs were atrophied. Despite this fact, the grafts were as well vascularized and as large as those which had maintained the condition of the endocrine organs in the previous experiments. Harris and Jacobsohn's paper also implies that the grafts were as satisfactory histologically as those under the median eminence, though the findings are based only on hematoxylin and eosin preparations and not on the special stains used for the other series.

Only two of the fourteen animals which received young pituitaries into the pituitary fossa showed estrous cycles, the endocrine organs were again atrophied. In this series, which is not discussed in such detail as are the others, the graft tissue was much smaller in mass and not so well vascularized. One might have expected these grafts to have been as successful as those under the median eminence.

Harris and Jacobsohn make two main points in discussing their results. They emphasize the difference between the effectiveness of grafts under the median eminence and that of grafts under the temporal lobe. Since both are equally well revascularized, they feel that a connection with the hypothalamic vessels is an essential factor in determining the function of a graft. They also draw attention to the speed with which very young pituitary tissue can take over adult function. Their belief that very rapid maturation of the grafted pituitary from a newborn animal takes place is not confirmed by May's observation (233) that diestrus in animals which received the transplants from newborn donors persists for 8 to 11 weeks before cycles begin.

Finally since male pituitaries can maintain normal cyclic changes in females, though they normally function at a constant level Harris and Jacobsohn feel that some higher center presumably in the hypothalamus, provides an essential rhythmic stimulus for the reproductive cycle in the female but not in the male simple interaction between pituitary and gonads does not seem to be a sufficient explanation.

Jacobsohn (1954) (255) cut the pituitary stalk of rabbits and transplanted grafts from the host's newborn litters under the median eminence. Many of the grafts were infiltrated by lymphocytes but a few seemed to be intact and were well vascularized from the portal vessels The hosts bearing such grafts had normal reproductive tracts and function Success in these instances is attributed to the graft and it is assumed that the host's own pituitary was rendered entirely functionless by the stalk section. The rapid maturation of young pituitaries, which was observed by Harris and Jacobsohn in rats, did not occur in the rabbits used in these experiments.

Jacobsohn and Jørgensen (1956) (256) have repeated the same type of experiment in toads. Autografts under the median eminence allowed the toads to survive for 6 months or more, and well differentiated pituitary cells with a good portal circulation were found. Grafts in the other situations were also well vascularized. The various types of cell could be distinguished but they were much less differentiated. Nevertheless, the animals died with all the signs of the deficiencies known to be caused by hypophysectomy They also carried out experiments with homografts. Though those grafts under the median eminence had some effect for a short space of time, grafts in either position underwent an immunity reaction and all the animals died.

### Effects of Pituitary Transplants

#### *Effect of Pituitary Transplants on Adrenal Cortical Function in Responding to Stress*

Cheng and associates (1949) (257) transplanted about a quarter of a rat's pituitary gland into the anterior chamber or the spleen of another rat, or they hypophysectomized the animal and immediately returned the gland to the pituitary fossa. Autografts were altogether more effective than homografts, especially when transplanted into the sella turcica. The eye was a more successful site than the spleen but in either site the grafts were incapable of supporting growth, and most of the hosts had atrophied testes and adrenals. However the grafts were able to discharge ACTH in response to stress, though in smaller amounts than normal. McDermott and associates (1950) (258) also transplanted tissue into the anterior chamber and found that the grafts retained the ability to secrete ACTH either spontaneously or in response to the local application of adrenaline to the anterior chamber The animals did not grow and the testes remained atrophic. The adrenals of the grafted animals weighed about twice as much as the hypophy

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calf pituitaries transplanted into the anterior chamber of the eye of either hypophysectomized or normal rats could find none.

Kylin (1938 1939) (278 279) treated twenty eight patients suffering from hypopituitarism with pituitary transplants. All the patients showed great improvement, which continued for two years or more. Westman (1940) (280) used the same treatment for a series of patients with primary or secondary amenorrhea who had not been improved by injections of ovarian or gonadotropic hormones. He was unable to ascertain whether the grafts had survived or not, but reports some success as a result of the treatment. Wolfsohn (1942) (281) found that these heterotransplants caused an intense local irritation in his eighteen patients and believes that the implanted organ had been resorbed within three months "Notwithstanding this fact the clinical effect persists, even for years." Westman (1949) (282) was satisfied that the cells of the transplant never survive Histologic examination of a single biopsy of a graft showed that it was completely degenerate He also emphasized the local inflammatory reaction mentioned by Wolfsohn and came to the conclusion that the implant acts as a form of shock therapy

Other clinical reports of the successful use of pituitary transplants are given by Kubanyi (1940) (283) in cases of Simmond's disease Arakawa and associates (1950) (284) in various dermatoses Watabe (1951) (285) in cases of alopecia, and Lang (1953) (286) in a wide variety of clinical conditions thought to be due to hypofunction of the pituitary

Implantation of pituitary tissue had no effect on the normal levels of ketosteroid excretion according to Borell, Diczfalusy and Westman (1952) (287)

**Relation of Transplanted Pituitary to the Development of Tumors**

Loeb and Kirtz (1939) (288) studied the effects of transplanting anterior lobe tissue on the growth of the mammary gland and on the development of mammary carcinoma in various strains of mice Transplants survived successfully for at least 8 to 10 months in the A, CBA and C3H strains. In the C57 strain there was often a little lymphocytic infiltration around the transplants. Increased activity in the mammary gland and an increase in the cancer rate were observed in those mice which had intact ovaries. The changes were similar to those induced by injections of estrogen and presumably indicate that the grafts stimulated abnormal activity in the ovaries. According to Silberberg and Silberberg (1949 1950) (289 291) grafts of the anterior pituitary also increased the proportion of animals developing malignant lymphoid tumors Silberberg and associates (1951) (290) showed that transplants of anterior pituitary in mice stimulated the growth of intrasplenic ovarian grafts and increased the proportion of intrasplenic tumors. The changes in the ovaries which normally occur with age were accelerated. According to Silberberg, Silberberg, and Opdyke (1954) (291) such transplants also increased the incidence and accelerated the onset of degenerative diseases of the joints.

If the thyroid of a mouse is destroyed by giving a dose of radioactive iodine tumors frequently develop in the pituitary (292) Such tumors are readily transplantable to mice whose thyroid glands have also been destroyed, but not to normal mice. The ovaries of mice bearing grafted tumors resemble those which had received pituitary hormones, and it is presumed that the tumor cells can produce not only TSH but also gonadotropin. The tumor cells which release the hormones are chromophobe in appearance and do not resemble the chromophil cells usually thought responsible for the formation of trophic hormones. After subpassage in thyroidectomized hosts one strain of tumor became able to grow in normal mice, where it caused tremendous hyperplasia of the thyroid (293) None of these tumors showed any signs of producing ACTH but another tumor occurring in mice which had been exposed to ionizing radiation seemed able to do so (294)

## ADRENAL CORTEX

Early workers on the transplantation of the adrenal cortex were concerned primarily with overcoming the many difficulties which seemed to prevent successful grafting (295) Failures were ascribed to something in the adrenal medulla (not adrenaline) which caused intense local irritation and destroyed the graft (296) For Cristiani (297) however who was as much a pioneer in this field as in the others, the transplantation of the cortex in rats presented no technical or histologic problems. He describes at length the histologic development of intraperitoneal grafts, how they become attached to

various abdominal structures, lose much of their cellular content and finally become fully restored in "une véritable restitutio ad integrum." These experiments were carried out on animals whose other adrenal gland had not been touched. Cristiani's difficulties began as soon as he removed this second gland even though it might be as long as a year after the first operation at a time when the microscopic appearance of the graft was fully restored to normal. Within a short space of time the animal died. To Cristiani the histologic picture of absolute adequacy and the equally complete functional uselessness of the cortical grafts, as shown by the death of the host, proved without question that the cortex was not concerned with the maintenance of life. He had noted that medullary tissue completely disappeared from the graft even when it was given every opportunity to acquire a new blood supply and came to the conclusion that the medulla must be the vital part of the adrenal gland. It is difficult to find fault with Cristiani's train of thought on the evidence he presented though we know now of course, that he was wrong. The difficulty lies in attempting to explain his observations. Even if all the graft's venous blood drained into the portal system, sufficient should have escaped inactivation to sustain life.

Busch, Leonard, and Wright (1908) (298) succeeded with a few autografts to the kidney but failed with grafts to other sites. The design of their experiments fulfilled the essential requirement—so often omitted—that removal of the graft (and of the other adrenal if it had been left in situ) should be followed by the signs of adrenal insufficiency and death. Blodinger, Klebanoff, and Laurens (1920) (299) also found the kidney to be the best site for autografts but their grafts failed to keep the dogs alive after removal of the second adrenal. More consistent success was achieved by Jaffe and Plavska (1926) (300, 301). Only four out of sixty-seven young rats died after adrenalectomy followed by transplantation. Jaffe (1927) (302) extended his observations to intramuscular grafts in guinea pigs and agreed with Elliott and Tuckett's (1906) (296) view that any difficulties with this species can be overcome by removing the medullary tissue before grafting. Fifty per cent of bilaterally adrenalectomized and grafted animals survived for more than 40 days. Jaffe also confirmed Cristiani's description of the immediate necrosis which overtakes much of the graft within three days of grafting; normal appearances were restored in four or five weeks.

Other workers at about this time (1928) (303–305) tried to transplant the gland either by use of the anterior chamber of the eye, or by means of pedicle grafting, but without success. These and the other failures can probably be ascribed to the speed with which animals succumb to the effects of adrenal insufficiency and to the feebleness of the circulation in an adrenalectomized animal, which makes it more difficult for a graft to reestablish vascular connections in time. Temporary treatment with cortical hormones or extracts was not available, nor had the life-saving properties of saline as drinking water for rats been fully realized. Pencharz, Olmsted, and Giragossintz (1931) (306) using rats and not the dogs so often used unsuccessfully by others, were able to transplant the cortex to the ovary but only, for some unknown reason, while the animals were in diestrus. A two-stage pedicle grafting operation to the ovary in the dog was developed by Dunphy and Keeley (1940) (307) and Keeley and associates (1940) (308). Levy and Blalock (1939) (309) used a vascular anastomosis technique by which the adrenal gland was transplanted together with the kidney to the neck of dogs. Their method was successfully employed later by Dempster (1955) (310).

Most of the significant work has been carried out on the rat. All workers are now confident that transplanting the adrenal cortex is perfectly feasible (see Turner (1939) (311) for grafts into the anterior chamber of the eye; Higgins and Ingle (1938) (312) for grafts into fascia surrounding the femoral vein; Pomerat, Breckenridge and Gordon (1944) (313) for grafts into the cerebral cortex) especially when the general condition of the host is maintained with cortical hormones and salt during the immediate post-operative period (fig. 187). Transplants of cortical tissue frozen in glycerol-saline, thawed 24 hours later are occasionally successful (Parkes 1955) (314) but the cortex is much less resistant to the effects of freezing than ovarian tissue.

### Functional Assessment of Graft

The most obvious and crucial test of the function of any adrenal gland graft is that it should protect the host against the effects of total removal of the adrenal glands and therefore a

TABLE 11

*Numbers of successful grafts of adrenal cortex according to host/donor relationship (from Ingle and Higgins (1938) (333))*

| Condition of Graft | Grafts Between | | |
|---|---|---|---|
| | Siblings | First cousins | Distant cousins |
| Gland viable—no lymphocytic infiltration | 13 | 7 | 4 |
| Gland viable—infiltrated with lymphocytes | 6 | 3 | 1 |
| Gland degenerated | 21 | 30 | 35 |
| Total | 40 | 40 | 40 |

of transplanted glands may be a function of genetic similarity and that the survival of homoplastic transplants could be used as an index for determining homozygosity in inbreeding experiments —a sentiment which should be entirely acceptable today

*Ingle Higgins and Nilson (1938) (332) report* further experiments on these two strains of rats which had been selectively inbred for efficiency in food utilization for about 20 generations. Grafts were exchanged between (*a*) 12 pairs of adult sisters from Strain I (*b*) 9 pairs of adult distant cousins from Strain I (*c*) 7 pairs of adult sisters from Strain II (*d*) 13 pairs of unrelated animals from Strains I and II. The host's adrenals were removed and the glands grafted to the ovaries cortin was administered for the first 10 days to give the grafts time to get vascularized. Forty-eight of the 56 animals with grafts from within their own strain survived with living grafts in the other eight rats the grafts degenerated and the animals died soon after grafting. More grafts showed signs of lymphocytic infiltration in the cousin-cousin grafts than in the sister-sister grafts. Of the 26 rats receiving grafts from the other strain 25 died of adrenal insufficiency the twenty-sixth had accessory tissue. Ingle and his coworkers conclude that close similarity in genetic constitution of donor and host is essential for regeneration and function of homografts A further report by Ingle and Higgins (1938) (333) provides the figures for table 11 The rats were all members of a Wistar strain which had not however been inbred

Everett (1940) (334) used male and female rats of the Long Evans and Sprague-Dawley strains to carry out auto- litter mate and interstrain transplantations. The autografts were successful and increased in size about threefold if the other host adrenal tissue was removed. About one quarter of the grafts between litter-mates succeeded, and only one in nineteen exchanged between *nonrelated rats was at all successful* There was no material difference in the proportion of 'takes' if the host's glands were removed, but only in the size of the graft and as Ingram and Krohn (1956) (159) found for the rat's ovary the removal of a single host gland did not help the survival of homografts.

In dogs Dempster (1955) (310) reports that adrenals which were homotransplanted (together with the kidney) by vascular anastomosis underwent a homograft reaction, as did the kidneys. (See also Kay 1952 (335))

The only discordant observations are those of Darcy (1952) (336) in the rabbit. He found that subcutaneous grafts exchanged between adult rabbits lived for many months and finally *succumbed to disuse atrophy rather than to any* homograft reaction. Surprisingly the chances of survival of the graft improved as the size of the graft was increased (See also Jones, 1951 (337) below)

*Methods of Abating Homograft Reaction*

Most of the available methods have been employed at one time or another but without much success.

(*a*) *Transplants to the brain* The brain is a favored site where the effects of a homograft reaction are usually mitigated but Longmire and Smith (1951) (338) report some others' unpublished experiments by Beale and Lockwood showing that *rat-rat homografts* and rat-rat heterografts to the cerebral cortex were unsuccessful.

(*b*) *The effect of cortisone and ACTH* Woodruff and Boswell (1953) (339) using Wistar rats as hosts and hooded rats as donors, transplanted whole glands to the ovary (the preferred site) or beneath the femoral vessels when day old glands were transplanted. In studying the effects of cortisone and ACTH on the survival of adrenal homografts, which would presumably be mediated by interference with immunity reactions, Woodruff and Boswell ran the risk, *which they recognized*, of having their results confused by the direct effects of the hormones

on their grafts They minimized the risk by using varying doses of cortisone and by giving injections of ACTH as a substitute growth stimulus for the endogenous ACTH whose production was inhibited by the cortisone. The histologic findings in the grafted adrenals after survival for 28 days or more were divided into four groups

1) Complete regeneration of cortical tissue Sometimes there was zonation but usually the whole graft resembled normal fasciculata. No inflammatory cells.

2) Extensive regeneration but increased connective tissue laid down and some degeneration of epithelial cells.

3) Some viable looking cells but much degeneration, and connective tissue formation. Numerous round cells or polymorphonuclear leukocytes.

4) Tissue disappeared or nothing viable.

Types 1 and 2 were regarded as successes, types 3 and 4 as failures.

The autografts were successful and the histologic findings in biopsies fell into types 1 and 2 Three untreated control homografts all failed to maintain life Of the homografts in treated animals roughly $^{2}/_{3}$ or $^{3}/_{3}$ were successful—figures which though they suggest that treatment may have had some effect, are not very encouraging. Williams (1953) (340) was also unsuccessful in the use of ACTH.

Using the general operative technique employed earlier by Levy and Blalock (1930) (309) for transplanting the adrenal cortex of dogs together with the kidney by vascular anastomoses to the carotid artery and external jugular vein, Kay (1952) (335) unilaterally adrenalectomized 37 dogs 4 to 18 days before homotransplantation and removal of the other adrenal. All the dogs died between the second and twentieth day after operation. There was no viable adrenal tissue at all except in the 20-day survivor Eight more dogs were given cortisone or cortisone and ACTH without improving the condition of the grafts.

Homburger and Bonner (1954) (341) transplanted human fetal material into six patients receiving ACTH as treatment for various skin diseases No surviving adrenal tissue could be found in any of the biopsies from the six patients at the end of several weeks treatment though the doses of adrenal hormone found necessary to control the diseases were lower than before treatment (see also p 440)

(c) *Tissue culture before grafting:* Another way of avoiding the homograft reaction is to use pieces of gland which have been maintained in tissue culture for some time before grafting Lux, Higgins and Mann (1937) (342) have grown adrenals from newborn guinea pigs and rabbits in media prepared from the plasma of the adult rabbits which were the future hosts. Eighty to 100 fragments of actively growing cells were transplanted to the groin after two to three weeks culture The hosts were not adrenalectomized. A connective tissue capsule was soon formed around the grafts and two weeks after transplantation lymphocytes were infiltrating through the capsule into the substance of the graft. Degeneration proceeded apace and the grafts had completely disappeared within 12 weeks. The reaction against the graft was a little delayed but Lux and associates thought that this was as likely due to the presence of the host's own adrenals as to a modification of the homograft reaction. Subsequently these same workers (1937) (343) homografted fragments of cultured newborn rat adrenal to unrelated adult rats which had been adrenalectomized. Lymphocytic infiltration destroyed 50 per cent of the grafts and to their mind any success was probably the result of genetic similarity rather than of changes induced in the fragment during culture.

Martinovitch (1955) (344) found that infantile adrenal glands which had been cultured for about 2 months grew better survived longer than normal transplants, and were able to maintain life in adrenalectomized hosts

(d) *Use of young donors:* The use of young donors was of some advantage according to Higgins and Ingle (1938) (312) Thus seven out of fifteen 2 week-old grafts were functioning 10 weeks after grafting compared with three out of fifteen 4-week-old grafts and one out of fifteen 8-week-old grafts. All the other grafts were destroyed by a typical homograft reaction. These investigators do not rule out the possibility that the 50 per cent survival is due to genetic similarity of host and donor—though they were taken at random from a colony that was not closely inbred and that came originally from a Wistar strain.

Jones (1954) (337) reports a series of experiments to study the merits of three different sites

for transplantation (the surface of the erector spinae muscle, within the muscle, and the femoral perivascular tissues) and of using newborn rat tissue. The adult hosts were adrenalectomized and maintained on salt for 2 to 3 weeks. In the 22 rats which survived out of 86 living grafts from the newborn donors were found which underwent growth until after 4 to 5 months, they were rather less in size than two normal adrenals. Removal of the grafts was followed by death of the host. Neither choice of site nor number of glands grafted (2, 3 or 6) altered the proportion of survivors. Of the 64 hosts which died 24 had grafts which were lying dead and inert in the tissues microscopically unchanged several weeks after grafting. They presumably failed to become vascularized. All the others had been destroyed. It is worth noting that hosts and donors came from rats bred in two different laboratories.

(e) *Effect of removing other endocrine glands:* One of the questions often brought up when the body's reaction to stress is under discussion, is whether the pituitary's production of the other tropins is affected by the increased output of ACTH which is clearly the main response. It is thought by some that the stimulus to the secretion of ACTH also releases extra quantities of gonadotropin. Others, pointing to the decreased genital function often seen in conditions of stress, suggest that production of ACTH can be increased only at the expense of gonadotropin. Similarly, if the adrenal cortex is removed more ACTH is produced, and if the ovary is removed, more gonadotropin. But does the production of the one affect the other (especially since they may be manufactured by the same type of pituitary cell) and what would happen if the two endocrine organs were removed simultaneously? Would the removal of the ovary increase or decrease the viability of an adrenal graft by altering the amount of ACTH that was available? Wyman and Tum Suden (1941) (345) have studied the effect of simultaneous gonadectomy and adrenalectomy on the proportion of successful homografts in their strain of inbred rats (25–30 per cent in nonsiblings). Their figures (table 12) seem to indicate that castration increases the proportion of survivors but only if it is carried out at the time of grafting. The effect has worn off in about 2 weeks. The fact that spaying, on the contrary, was probably ineffective makes one a little doubtful about the specificity of the response in the group of males used.

TABLE 12

*The proportion of successful transplants of adrenal cortex in normal, castrated and spayed rats (non siblings within a strain) (from Wyman and Tum Suden (1941) (345))*

| Description of Hosts | Successful Transplants | |
|---|---|---|
| | Proportion | % |
| Normal control males | 9/28 | 32.1 |
| Castrated at time of homotransplantation males | 21/39 | 53.8 |
| Castrated 2 weeks before homotransplantation males | 4/10 | 40.0 |
| Castrated 2 to 3 months before homotransplantation males | 12/58 | 20.7 |
| Normal control females | 16/64 | 25.0 |
| Spayed at time of homotransplantation females | 11/36 | 30.5 |

## Validity of Halsted's Law

Wyman and Tum Suden (1932) (346) attempted unsuccessfully to supplement the amount of circulating adrenal cortical hormones by transplanting extra adrenal glands. Although the grafts regenerated rapidly in adrenalectomized rats to about a constant total mass which was greater in the female than in the male, they would not grow in the presence of even a fragment of one of the animal's own glands (see also Higgins and Ingle, 1938) (312). Ingle (1950) (347) confirmed this finding and showed that such grafts would also fail to respond to stress by an increase in size, but he made it clear, in addition, that the graft was nevertheless still viable—as is an ovarian graft in similar circumstances.

Levy and Blalock (1939) (309), using their method of vascular anastomosis, showed that an adrenal gland can be transplanted without difficulty even in the presence of the remaining contralateral gland, and will maintain the health of the host subsequently when the other gland has been removed. Similar findings have been reported by Dempster ([illegible]) (310) who emphasizes especially the differences to be expected between the results of vascular anastomosis with immediate restoration of blood flow and of implanting the adrenal into a pocket in muscle or connective tissue where some delay is in

evitable before the gland obtains a new blood supply

It has been mentioned earlier that explanations for the difference in behavior of grafts according to the presence or absence of host tissue are usually given in terms of the production of trophic hormones by the pituitary Wyman and Tum Suden (1937) (348) studied the behavior of cortical grafts in hypophysectomized animals, which should be unable to produce the normal stimuli on which growth is said to depend Using a strain of rats in which nonsiblings can often exchange grafts successfully they found that 71 per cent of females (but only 20 per cent of males) gave successful grafts in control experiments. Such homografts always failed in hypophysectomized animals whether they were adrenalectomized as well or not. Rats which had been hypophysectomized three months earlier died within a few days of combined adrenalectomy and autografting Atrophied glands from hypophysectomized animals could be grafted successfully They believed therefore, that it is the amount of pituitary hormone actually available to the graft (after preferential take-up by the established tissue) rather than a "need" or an adrenal deficiency which controls the amount of regeneration. Their failure to establish autografts may be related to the rapid death from adrenal insufficiency before one could hope for any graft to have established itself rather than to the absence of ACTH which would have been unable to reach the graft in any quantity soon enough.

Turner (1939) (311) found that the extent of regeneration and the frequency of successful grafting to the anterior chamber of the eye were increased not only by adrenalectomy but also by the implantation of pituitary tissue. But proliferation did occur even if the host's adrenals were untouched. Turner therefore felt that either there is normally more circulating ACTH than is required by the host's own glands, or there is no preferential take-up of ACTH. These facts conflict with the consensus about the grafting of ovarian tissue, but add further weight to the belief that Halsted's law cannot be applied rigorously

According to Higgins and Ingle (1938) (312) glands from newborn rats grafted to the fascia covering the femoral veins do not regenerate to any extent in the presence of the host's own glands, but the total amount of regenerated graft (in adrenalectomized hosts) is rather more than the combined weights of the host's original glands. Silberberg Silberberg and Opdyke (1953) (349) seem to have established some sort of ratio between the amount of pituitary tissue and successful adrenal grafts. Extra pituitary grafts stimulated extra adrenal growth in up to four extra adrenal glands.

### Regeneration of Tissues after Grafting and Origin of Cortical Cells

One of the main problems in adrenal cortical physiology—and one amenable to the technique of transplantation—has been to determine the site of origin of the cells which make up the gland and the life span of the elements of the zones into which it can be morphologically divided It has been thought by many that only the thin layer lining the capsule is able to form new cells and that there is constant centripetal migration of cells from this area. It is certain that an entire adrenal cortex will regenerate if the capsule alone—after enucleation of the rest of the gland—is transplanted.

The sequence of regenerative changes in the first 21 days after grafting has been described by Brenner and associates (1953) (320) Autografts were made into the muscles of the back of totally adrenalectomized rats, which were maintained on saline drinking water Three days after grafting degenerative changes were widespread, and many nuclei did not stain at all. Macrophages and polymorphonuclear leukocytes had entered the transplants and were phagocytosing lipid droplets released from the dying cells. Reabsorption was complete within about 12 days. These workers describe a sequence of five cell types during regeneration from the surviving fringe of glomerulosa cells (but not from the capsule) which leads to a reformation and differentiation of the cortex complete except for the restoration of a lipophobic zone. Descriptions of the reparative and regenerative processes in dogs and rats are also given by Dempster (1955) (310) The responses of these two species differ in the extent to which zonation is restored

It has already been pointed out that maximal protection against some forms of stress is provided by a 10-day-old graft. At this time the cells are empty and contain no lipid droplets Presumably there is no time for storage of hormones, which are all required by the body as soon as they

are produced. Storage of secretions comes a little later: fine granules appearing in the cells on the eleventh to fourteenth days.

Williams (1945) (350) has studied the behavior of grafted cortical cells in transparent chambers in the ears of rabbits for periods of up to eight months. Glomerulosa cells appear to become vascularized more rapidly than do fasciculata cells, and go through a slow cyclic change of discharge and accumulation of granules that is accomplished without loss of numbers. The number of fasciculata cells, on the other hand, steadily decreases. Williams (1947) (351) reports that, after a long postoperative period, the original fasciculata and reticularis zones disappear. He is confident that the glomerulosa is the only source of new cells which become organized to form new but imperfect fasciculata and reticularis zones.

Coupland (1955) (352) who inserted pieces of adrenal cortex into the anterior chamber of the eye reports, however that typical glomerulosa cells may be formed from a graft that was originally made up entirely of reticularis cells. He argues, therefore, that the reticularis is an active, growing zone as much as any other and that zonation is simply a positional effect. He points out incidentally that mitoses can be observed in the grafts even when the host's other adrenal is undisturbed.

### Transplantation of Adrenal Cortex in Clinical Practice

Numerous attempts have been made to transplant pieces of adrenal cortex—often from newborn donors or from patients with hyperactive adrenal glands—to patients suffering from Addison's disease. Bailey and Keele (1935) (353) report such a case in which rather surprisingly the response to the implant was considered to be better than to cortical extract. Four years later they reported (354) the continued success of the treatment but pointed out that, though the diagnosis of Addison's disease was accepted by the meeting at which the patient was first demonstrated, there could be no certainty of this nor had it been possible to make any biopsies of the grafts.

Katz and Mainzer (1941) (355) reviewed the earlier literature and came to the conclusion that not more than five cases could be judged to have been successful. They submitted a sixth case for which they believed that their evidence for success was adequate. Simpson (1941) (356), in a skeptical discussion of Katz and Mainzer's report, considered that grafts were, at the best, of temporary value only. Thiersch (1943) (357) grafted fetal tissue into the intrasternal bone marrow and found that the dose of cortical hormone necessary to maintain the patient in normal health could be lowered.

The difficulties inherent in clinical experiments of this kind are brought out by Hunt's (1941) (358) case. The man, accepted as a typical case of Addison's disease, was treated by transplantation of neonatal cortex into the testis. Twenty-seven months later he died, apparently of Addisonian anemia. But at autopsy absolutely normal adrenal glands were found and no trace of grafted tissue.

Homburger and Bonner (1954) (341) transplanted fetal cortical tissue into patients who were receiving treatment with ACTH. Despite this added growth stimulus they were unable to recover any grafts at biopsies carried out several weeks later. All six patients nevertheless improved.

In attempting to explain these results Homburger and Bonner suggest that the transplants must in some way improve the performance of the patient's own glands which, unlike those of patients with Addison's disease, are presumably able to function normally. They quote an unpublished communication by Greene who transplanted a fetal thyroid gland into a myxedematous patient. The iodine uptake and protein-bound iodine level improved after the operation but none of the transplanted gland could be found at biopsy. These apparently nonspecific instances of clinical improvement recall other clinical remissions in rheumatoid arthritic patients after absorption of heterotransplanted calf pituitaries.

Woodruff (1952) (359) finds the results of successful vascular anastomosis of a hypertrophied gland reported by Broster and Gardiner Hill (1946) (360) hard to understand. He himself made intramuscular transplants of fragments of fetal adrenal gland to four patients with Addison's disease. Cortisone and ACTH were administered for four to five weeks in an attempt to delay the onset of an immunity reaction. All the patients showed striking clinical improvement, which was maintained for more than a year

Thorn tests, however showed only temporary improvement and biopsies in three cases, 6 to 9 months after operation, showed no trace of the transplants. Woodruff concludes "In face of the negative biopsy, finding of clinical improvement remains a mystery

Summers and associates (1957) (361) suggest that when treatment of hormone-dependent carcinoma of the prostate requires adrenalectomy to assist in reducing the circulating androgen, it would be worth while transplanting the excised cortical tissue to the mesentery whence its hormones would pass into the portal circulation. Any androgen would be destroyed by the liver while life-maintaining corticosteroids would pass through reasonably intact. The need for supportive therapy with cortisone would thus be avoided. Other workers have tried anastomosing the veins draining the adrenal gland to the splenic vein, but Summers and coworkers preferred to transplant the glands in two patients whom they adrenalectomized The glands were freed of medullary tissue, cut into thin slices, and placed in pockets in the transverse mesocolon. The patients still required substitution therapy and the response to test doses of ACTH was equivocal. At autopsy 6 and 7 months later small healthy looking but inactive masses of graft tissue were found. Summers and associates (1957) (361) based these clinical trials on experiments on dogs, using similar techniques (362) In none of the 26 dogs used did the grafts maintain life after cortisone therapy was withdrawn. It is, therefore, not very surprising that their clinical experiments were rather unrewarding. Either a large proportion of the corticosteroids are destroyed in the liver or the technique of autotransplanting in human beings needs to be improved Bernstein Brikind and Lincoln Brown (1955) (363) on similar grounds autotransplanted the adrenal cortex into the mesentery in 16 women with metastatic carcinoma of the breast. Once again evidence for function was at best equivocal.

### Transplantation and Development of Tumors in Adrenal Cortex

It has already been pointed out that the general technique of transplantation can be of great value in gerontologic studies for it is the only method of studying the behavior of organs of one age in the environment of another age. It is also of equal value wherever one wishes to examine the behavior of organs from two strains in a common environment. The technique has been exploited by Huseby and Bittner (1948) (202) in their studies on the carcinogenicity of ovarian grafts (see p 421) They have also (364) applied it to studying whether the strain differences in the responses of mouse adrenals following early gonadectomy are due to differences inherent in the adrenal tissue itself Of the three strains, A, Z and CE, the A strain responds hardly at all, the adrenals of the Z strain hypertrophy and secrete female sex hormone only while CE adrenals become carcinomatous and secrete both male and female hormones. These characteristic responses of the strains persist when the glands are transplanted into $F_1$ hybrid recipients though they take longer to develop than usual. For example, Z adrenals transplanted to Z × CE $F_1$ animals became hyperplastic while CE adrenals in the same environment became carcinomatous.

## *ADRENAL MEDULLA*

It is often stated that the neurohypophysis and the adrenal medulla are the two endocrine glands which cannot be transplanted successfully Failure has been correlated with their embryonic derivation from neural tissue and their dependence on a nerve supply This general opinion appears to be true of the posterior lobe of the pituitary (*e g* Siperstein and Greer, 1956) (246) but is certainly not true, despite some difference of opinion, for the adrenal medulla. On the one hand, we find that Williams (1947) (351) failed to get grafts to grow in the ear as did Bernstein (1950) (323) using the spleen or mesentery as the site. Jaffe and Plavska (1926) (300) and Weinstein, Schiller and Charipper (1950) (328) also reported that the medulla does not survive transplantation. On the other hand, Busch Leonard and Wright (208) were successful in two transplants to the kidney as far back as 1908. Keeley and associates (1940) (308) were also successful in a two-stage pedicle graft to the ovary and reported that bioassays showed adrenalin-like substances in the graft. Levy and Blalock (1939) (309), who had transplanted the gland by vascular anastomosis to the neck, could not, however demonstrate any adrenalin in the venous effluent from the transplants. Turner Haffen and St. Amant (1939) (305) were consistently successful in transplanting the medullary tissue of whole

newborn adrenal glands under the kidney capsule of the mother provided that early vascularization was achieved by bringing the hilum of the gland into close appoeltion with the kidney. Transplants of teased medullary tissue from which the cortex has been removed were unsuccessful. Huseby and Bittner (1951) (364) found normal medullary tissue as long as 12 to 17 months after grafting in 93 out of 127 grafts in mice. Finally Coupland (1955) (352) has separated the medulla from the cortex and reports satisfactory survival of grafts in the anterior chamber of the eye.

Everett (1949) (334) not only reports successful autografts of the medulla but also mentions that homografts show the cellular infiltration characteristic of the response to homografts of other organs.

## THYROID

It is appropriate here to emphasize the considerable and largely overlooked contribution of Cristiani to the study of transplanted endocrine glands. Not only was he concerned around the beginning of the twentieth century with working out the optimal conditions for the growth of autotransplants, but he was also interested in studying the responses to a wide variety of homotransplants and heterotransplants. In 1895 he (366) gave detailed histologic descriptions of the development of grafts placed in the peritoneal cavity which differ little from the more recent descriptions by Dempster and Doniach (1955) (367) for subcutaneous grafts. At that time there was some confusion as to the relative importance of the thyroid and parathyroid glands. Cristiani believed that removal of the thyroid was fatal for he did not realize that death was actually due to concomitant removal of the parathyroid in a species (like the rat) which has the parathyroid glands embedded in the thyroid tissue. The fatal result, he found, could be prevented by an autograft which functioned for as long as the normal life span of the rat. Cristiani (1903) (368) grafted very small pieces of thyroid tissue into an ear of young rats and watched the grafts for one to two months. By this time they were extremely small but were still living for as soon as he removed most of the rest of the animal's thyroid tissue the small graft reddened and increased rapidly in size.

Cristiani and Cristiani (1905) (369) investigated the effect of grafting young thyroid tissue into older animals. The donors were about three months old and the hosts were either of the same age or between one and two years old. The grafts into young animals "took" best, while the grafts into the old animals did not regenerate completely. The differences between the responses of young and old animals were not, however, striking. Cristiani (1903) (370) also attempted to find out how often grafts could be transplanted from one host to another. Grafts that were removed 8 to 13 days after the first transplantation were unable to survive subsequent transplantation but those which had remained in their new site for a month or more could still be found after the second transplantation and had a perfectly normal histologic structure. Other much more recent workers (371-373) have reported similar experiments. Cristiani (1905) (374) clearly formulated the principle subsequently laid down by Halsted

> It can be concluded that if there is to be reconstitution and progressive evolution of the thyroid graft it is necessary for the organism to feel a want of thyroid function either because its own thyroid gland is absent or sick or because it is quantitatively insufficient. The development of the graft will be proportional to the need of thyroid felt by the grafted organism

While this may be true of the "evolution" of the graft, Cristiani's own observation (1903) (368)—that the small pieces of thyroid tissue were viable and remained ready to respond to the change in their environment caused by complete thyroidectomy—had already shown up the limitations of the generalization.

Most workers are satisfied that the nerve supply to the thyroid is unnecessary for normal responses, since they have found that thyroid tissue no matter where it was grafted, retains a normal histologic appearance and responds to treatment with iodine by the usual process of involution (375). Others who believe that the grafts behave entirely normally without nervous connections include Kummer (1917) (376), Akamatsu (1923) (377), Marine and Rosen (1934) (378) and Weil and Bernheim (193 ) (379).

Hirschberger (1931) (380) however believes that the return of sensitivity to injected anterior pituitary hormones which begins about 7 or 8

lays after grafting (at the time of revascularization) is related to the formation of new connections with the nervous system Kühne (1952) (381) reached the remarkable conclusion that autotransplants into muscle ultimately disappear, whereas those transplanted close to the cut end of the vagosympathetic nerve in the rabbit retain a reasonably normal structure. He regards this as being due to the influence of acetyl choline produced locally by the nerve fibers.

## Functional Assessment of a Graft

In recent years the assessment of the function of a graft of the thyroid has been facilitated by the readiness with which the uptake of radioactive iodine can be measured. Bennett and Gorbman (1951) (382) used male mice whose own thyroids were undisturbed, and grafted additional thyroid lobes from donors of the same strain sex, and age, under the skin on the top of the head. Strongly positive radioautographs were obtained as soon as 6 hours after grafting, but no way could be found to distinguish morphologically between transplanted tissue able to metabolize iodine and those cells which were unable to do so These early positive radioautographs were obtained when necrosis of of the graft was greatest and before any new vascular connections had been established. A little later when recovery was beginning and vascular connections had been restored, the function of the grafts, as tested by radioiodine uptake was at its lowest level. Restoration of function was almost complete by 10 days and fully restored 21 to 40 days after grafting According to Marnoni and Bonatelli (1953) (383) autografts always take up radioactive iodine. Brücke and associates (1955) (384) found that autografts into the anterior chamber of the eye took up radioactive iodine normally as do autotransplants subcutaneously (385) Wollman and Scow (1955) (386) reported that, although autotransplanted lobes were always smaller than lobes *in situ* their clearance of radioiodide from the blood is no different per unit weight of tissue and they do not differ in their response to hypophysectomy or feeding with propylthiouracil.

"Homografts" often take up radioiodine especially in thyroidectomized animals (383) but heterografts never do Aron and associates (1935) (387) have used the iodine uptake method to study the function of homografts transplanted into the testis of guinea pigs. Minute thyroid fragments were still fixing large quantities of iodine 6 to 16 weeks after grafting and responded to TSH or thyroxin.

Practically all the work on thyroid transplantation has employed the usual technique of subcutaneous or intramuscular transplantation. There have been only a few attempts at direct vascular anastomosis. Carrel and Guthrie (1905) (388) succeeded in removing the right thyroid gland of a dog and replacing it in the neck, anastomosing the superior thyroid artery to the jugular vein, and the thyroid vein to the carotid artery The grafts were inspected 11, 20 and 55 days after operation and seemed to have taken satisfactorily Kawamura (1919) (389) employed the method successfully in four out of eight autografts that he attempted in dogs. None of the seven homografts was successful (see also Hess and Strauss, 1917 (390))

## Fate of Thyroid Grafts (Especially Homografts) (fig 189 190)

Many of the papers which follow Cristiani's pioneering observations deal with both auto- and homografts together Hesselberg (1915) (391) in a detailed report on the histologic appearance of auto- and homografts removed from the subcutaneous tissue or abdominal musculature one to 50 days after transplantation in guinea pigs, recognized three stages in the life of the transplant (see also Loeb 1930 (392), Ingle and Cragg 1939 (393) see also Williams, 1937 (394) for long term observations on grafts in transparent ear chambers) For the first four or five days there is no noticeable difference between autografts and homografts. As Cristiani had found earlier large parts of each sort of graft become necrotic in the center leaving only a narrow zone of viable thyroid tissue around the periphery Mitoses begin to appear after about three days, and new follicles are forming after four days. During the next week the differences between auto- and homografts become very obvious Large numbers of lymphocytes and fibroblasts infiltrate the homografts whose follicles are rapidly destroyed, until after about 30 days no homograft tissue can be seen. In the meantime the regeneration of autografts is virtually completed

Manley and Marine (1916) (395) transplanted thyroid tissue to all parts of the body in a large



FIG. 189 (*left*). A thyroid isograft (mouse) eighteen days after grafting. Note central necrosis and reformation of thyroid follicles at the periphery of the graft. ×100.

FIG. 190 (*right*). A thyroid interstrain homograft (mouse) eighteen days after grafting. The thyroid follicles are still recognizable but are surrounded and being infiltrated by round cells. ×75.

series of 507 homotransplants to 205 rabbits of various ages. Complete absorption of the graft might take place as early as the tenth day and usually occurred before the thirtieth day. In their entire series only two or three rabbits accepted homografts, and in 92 per cent of them the grafts were destroyed in from 10 to 30 days. Goodman (1916) (390) and Hess and Strauss (1917) (390) were also satisfied that homotransplants (grafted by means of blood vessel anastomoses) did not survive for more than 2 to 4 weeks in dogs.

The following quotation from Manley and Marine clearly indicates their recognition that repeated homotransplantation accelerates the destruction of homografts, an observation which forms one of the crucial tests of the immunologic basis for rejection of homografts. "As is well known, repeated homotransplantation markedly accelerates the destruction of homografts due to the development of an immunity, the nature of which is little understood."

Hesselberg and Loeb (1918) (397), recognizing that the lymphocytic response to a second transplant of a tumor is accelerated, wished to test whether the same acceleration of reaction would occur in response to repeated thyroid transplants. They transplanted one thyroid subcutaneously and 9 and 11 days later another lobe from a third guinea pig was transplanted into the same host. The lymphocytic reaction against the graft was not accelerated though occasionally it seemed to be somewhat stronger around the second than around the first graft. Unfortunately the design of their experiments, which led them on several occasions (e.g. Loeb, 1930, 1945) (392, 398) to deny the cardinal fact that demonstrates the general immunologic nature of the homograft reaction, cannot be regarded as satisfactory. The second set reaction is so specific that the use of the same donor for the second graft as was used for the first (or of an isogenic donor) is absolutely essential. Where another donor is used one can rely only on fortuitous similarities to provide an accelerated reaction.

Loeb (1918) (399) transplanted lobes from several animals simultaneously and found that the speed of the reaction to the individual lobes varied, although the end result was destruction of all the grafts. The results of transplanting thyroid tissue between closely related animals were intermediate between those obtained with autotransplants and those with homotransplants (400). The transplants usually behaved like autotransplants to start with but in most cases were ultimately destroyed by an intense lympho-

cytic infiltration the rapidity with which this effect occurred depending on the closeness of the relation between host and donor Loeb (1926) (401 402) found this to be true for both guinea pigs and rats.

Loeb and Wright (1927) (403) demonstrated the genetic homogeneity of their stock of guinea pigs by successful exchanges of grafts of thyroid tissue between pairs. On the other hand, Loeb and King (1927) (404) found that their stock of rats had not approached homogeneity despite forty generations of inbreeding. They pointed out, however that the inbreeding of their rats was selective for the strongest animals and may have been unintentionally selected for heterozygous vigor while, on the other hand, the guinea pigs for each new generation were chosen at random. Another twenty generations of inbreeding of the rats had improved the results but differences between transplants to litter mates and those within the strain could still be detected (371) The approach to homozygosity was accelerated from the sixty-second to the one-hundred and seventh generation of brother-sister mating, but complete homozygosity had not even then been achieved (405) Not only the thyroid but also the ovary was used as a test graft in these experiments. The two endocrine organs behaved very similarly though the thyroid was perhaps a little less resistant to the homograft reaction.

Salmon and Severinghaus (1936) (406) studied a series of thyroid transplants in rats of the Long Evans strain which had been inbred for 23 years. They found that homografts between day old litter mates and from day-old rats into thyroidectomized older rats were successful a month later as judged by the rate of body growth and the histologic appearance of the grafts. They regarded their results as indicating complete compatibility within the strain and continued "The next stages in the experiment will be attempts to transplant the infantile rat thyroid into rats of an unrelated strain and if these attempts are successful heterografts will then be attempted. The results of these experiments, if they were ever carried out, do not seem to have been reported

Woodruff and Woodruff (1950) (90) have made one of the most complete studies of the results of auto- and homografting thyroid tissue in guinea pigs (see also Dempster and Doniach, 1955) (307) Autografts were always successful, either in the anterior chamber of the eye or subcutaneously provided that the remainder of the host's thyroid had been removed. Homografts were successful in 78 per cent of cases when grafted to the anterior chamber but only in 11 per cent when grafted subcutaneously The homografts were definitely antigenic for a second graft broke down more rapidly than the first. Similarly, the immunity induced by a subcutaneous homograft was general and extended to the anterior chamber where destruction of a second graft, after the earlier subcutaneous graft had broken down, was accelerated.

The main fact which must be discussed in considering Woodruff and Woodruff's work is their demonstration that the anterior chamber of the eye is such a favorable site Medawar (1948) (89) has suggested that skin grafts survive in the anterior chamber only as long as they remain unvascularized. As soon as they can be reached by blood-borne factors they are subject to the homograft reaction. But Woodruff and Woodruff are at pains to point out that the grafts survived in the anterior chamber despite becoming vascularised their survival did not depend on *quasi* tissue culture conditions. Perhaps the important condition which affects the fate of the graft is not so much the extent of revascularization as the amount of antigenic material which is able to pass from the graft into lymphatic channels. The acquisition of a new blood supply by a graft in the anterior chamber does not necessarily imply that antigens reach the regional lymph nodes in sufficient quantities to be effective since the anterior chamber has no lymphatic drainage as such. When Woodruff and Woodruff brought reticuloendothelial tissue close to the graft by adding a spleen autograft to the anterior chamber the thyroid homograft had very little chance of survival. Presumably destruction of the homograft followed a release of cytotoxic material produced locally in response to liberated antigens.

Woodruff and Woodruff go on to show that a graft which has maintained itself successfully for several months in the anterior chamber is no longer sensitive to a subcutaneous transplant from the original donor which, if made at an earlier stage would have been expected to cause rejection of the graft in the eye They felt that this fact implied a progressive fall off in vulnerability and that after a certain so-called "critical period" a graft becomes better able to withstand an immunologic attack Since there is no evidence that the amount of antibody formed in

to a continued stimulus diminishes their view implies some form of adaptation of the graft to it new environment. Similar changes whereby a grafted tumor becomes less antigenic perhaps by selection of compatible strains of cells have been recognized, and it may be that the components of those grafts which survived for several months in the anterior chamber of the eye had, in effect, been selected for genetic compatibility It could also be argued that the results are inherent in the design of the experiment, for if any particular first-set graft survives for a considerable length of time it must be presumed to be compatible and therefore unlikely to be affected by a second graft.

It is quite clear that the immediate result of grafting depends on how well the graft can put up with the absence of a blood supply and how quickly a new supply develops. Both these factors are independent of circulating trophic hormones and it is not until a new circulation has been established that modifications of the hormonal environment can affect the growth of the graft. Merwin and Hill (1954) (407) have used a transparent chamber technique to make observations on the way in which subcutaneous homografts of thyroid tissue are vascularized. They found that those which were vascularized within a week were usually destroyed within 12 days of receiving a new blood supply Nonvascularized homografts, however survived indefinitely unless a vascularized graft from the same donor strain was, or recently had been, present in the host. That is to say nonvascularized subcutaneous grafts were unable to elicit an immunity reaction but could be destroyed by an existing immunity If such homografts were not vascularized until the third to fifth weeks after transplantation, they survived for at least two weeks longer than grafts which had been vascularized early Their power to initiate an immunity reaction seemed to have become less effective Merwin and Algire (1956) (408) found that the vessels of thyroid grafts form buds but that only a few of these join up with the host vessels. Unlike tumor tissue the thyroid grafts do not stimulate the formation of a network of surrounding host vessels.

Both Cristiani (1901) (409) and Loeb (1920) (410) reported that heterotransplants were totally unsuccessful.

*Mitigation of Homograft Reaction*

(a) *Transplantation to brain.* Attempts to mitigate the homograft reaction have made use of the usual devices Thus Siebert (1925) (411) used homotransplantation to the brain in the hope that the lymphocytic reaction would be abated there The homotransplants did, in fact, survive considerably longer than normal, but lymphocytes still managed to infiltrate the grafts and the results were not as good as those obtained with autotransplants. There was considerable connective tissue reaction.

(b) *Effect of treatment with hormones:* Silberberg (1934) (412) who found that extracts of anterior pituitary tissue induced hypertrophy and hyperplasia in the autotransplanted gland also found that treated homotransplants were more active and better able to survive provided that a stimulus to growth was not also given before transplantation. The stimulated gland appeared to be less suitable for transplanting than the resting normal gland Treatment with thyrotropin, on the other hand, had no effect according to Woodruff (1954) (413) and Dempster and Donach (1955) (367)

Williams (1953) (340) using his transparent chamber method (previously used Williams 1937 1939 (394 414) to study the development of thyroid follicles in grafts) found that neither ACTH nor cortisone had any effect in prolonging survival.

Woodruff and Boswell (1954) (415) transplanted homografts of thyroid tissue subcutaneously in guinea pigs which were treated with Phenergan. The proportion of successful grafts at both 4 weeks and 4 months was increased in the treated group and nearly or just reached a statistical level of significance. Success was possibly due to a stress response which increased the amount of cortisone secreted by the host, for Woodruff (1954) (413) has found, too, that large doses of cortisone may facilitate survival of thyroid homotransplants.

(c) *Treatment with injections of blood:* Preliminary injections of blood from recipient to donor rabbits or of an extract from the donor tissue usually made matters worse according to Hess and Strauss (1917) (390) and to Hegard (1938) (416) no matter whether the blood was given in single or multiple doses Administration of blood from an animal other than the donor of

the graft had no effect at all. The rate of destruction of heterotransplants was always quite unaffected.

Shafiroff and McCloskey (1937) (417) exchanged 36 transplants in dogs with compatible blood groups. None of the grafts took, and after 3 to 4 weeks thyroid tissue could only just be identified, extensively infiltrated with round cells.

(d) *Effect of age of donor.* Loeb (1926) (401) found that the typical homograft reaction was less well developed in young hosts than in old ones. He also noted that the reaction was less pronounced during the early stages of pregnancy in normal animals. Modifications of the reaction to skin homografts in pregnant rabbits have been reported by Heslop, Krohn and Sparrow (1954) (418) and attributed by them to the extra production of adrenal cortical hormones at that time.

Tureen (1927) (419) compared the reactions of adult and young guinea pigs against homotransplants. He found that the reaction was less intense in the young animals than in the adults, and goes as far as to suggest that homotransplants into young animals may sometimes resemble syngenesiotransplants into adults. However, it is evident from his paper that he expected all homotransplants to break down fairly rapidly.

May (1933, 1936) (420–422) reported that brephoplastic homotransplants of the thyroid, unlike adult tissues, appeared histologically normal for up to 490 days after grafting. Dameron (1953) (423) transplanted fetal rabbit thyroid tissue to the anterior chamber of the eye. Six homografts concentrated radioactive iodine normally and were stimulated by TSH but not by propylthiouracil. Bariatti (1953) (424) has also used brephoplastic grafts successfully in the anterior chamber of the eye of guinea pigs when grafts of adult tissues failed.

### Validity of Halsted's Law

It has already been mentioned that Cristiani (1905) (374) clearly formulated the principle subsequently laid down by Halsted though he had earlier also provided the sort of evidence (of survival of fragments where there was no hormonal deficiency) which may be said to disprove the law's absolute validity.

A further test of the general hypothesis is to graft extra glands to animals which have their full normal complement. Under such circumstances the grafts should fail because their secretions are not required. Loeb and Hesselberg (1919) (425) found, however, that extra grafts were acceptable, their fate clearly did not depend on the physiological need for the thyroid hormone. Loeb (1930) (392) grafted as many as four extra lobes with success. Bennett and Gorbman (1951) (382), as has already been mentioned, found that extra thyroid lobes would take up radioiodine.

Further evidence on the validity of Halsted's law is provided by Ingle and Cragg (1939) (393) who studied the regeneration of autografts of thyroid tissue in partially or completely thyroidectomized rats. Although regeneration was more exuberant in completely thyroid-deficient animals, it still took place in animals with part of their thyroid tissue intact.

Woodruff and Woodruff (1950) (90) accepted the basic correctness of Halsted's law (as extended to cover homografts) as far as grafts in the eye are concerned, for they found that the chances of obtaining a successful homograft were proportional to the amount of host thyroid tissue removed. But autografts either subcutaneously or into the eye showed no such consistent relationship and certainly autografts took just as well with a 50 per cent deficiency as with total deficiency of thyroid tissue.

Liddle, Wittenstein and Swan (1954) (426) found that autotransplants took satisfactorily in a variety of situations in dogs, provided that the transplants were not more than 3 mm. thick and the animal had been thyroidectomized. Their significant contribution is that the grafts were equally successful in animals which had been hypophysectomized as well and which therefore had no extra output of thyrotropin to account for success. (This result was true of the adrenals as well, though the over-all success rate was rather smaller.)

Dempster and Doniach (1955) (367) provide the most recent comprehensive account of the survival of thyroid transplants, especially in relation to Halsted's law. They have attempted to answer the questions: "Do autografts take in animals without thyroid deficiency? Is there any correlation between degree of deficiency and proportion of successes?" and "Does the degree of deficiency cause a survival of homotransplants?"

Rats receiving autografts were either quarter half or totally thyroidectomized, and some half thyroidectomized animals were given thyroxine in sufficient quantities to suppress the normal production of thyrotropin. An extra thyrotropic stimulus was provided by giving a further group of animals methyl thiouracil. Function of the grafts was assessed by the uptake of radioactive iodine. Uptake was measurable 10 days after grafting although it was still low; it was increased by giving thiouracil.

Recognizable thyroid tissue was found in 25 out of 69 homografts, but only 13 out of 42 survived 10 to 20 days or more. Six days after grafting homografts still looked like autografts but by 10 days they were heavily infiltrated with lymphocytes and were soon destroyed. The administration of methyl thiouracil, which should have increased the amount of thyrotropic hormone available to the homografts, had no effect on their chances of survival, nor had the extent of the thyroid deficiency (see also Marine and Rosen, 1934) (378). Dempster and Donnach's work provides convincing evidence that the success or failure of grafts, whether autografts or homografts, is quite unrelated to the degree of thyroid deficiency that is produced.

### Effect of Site of the Graft

It is known that much of the circulating thyroid hormone is normally localized in the liver and excreted in the bile. Direct drainage to the liver of all the blood from an intrasplenic graft might therefore affect the transplant by an alteration in the pituitary's production of trophic hormone similar to that which influences intrasplenic grafts of the ovary. Experiments have shown that any such effect of the liver is only partial—a result which is in line with the fact that thyroid hormone is active even when given by mouth.

Bondy (1951) (427) totally thyroidectomized rat and autotransplanted one lobe into the spleen. The grafts maintained growth of the rats and histologically were no different from normal, a finding which Bracheto-Brian and Grinberg (1951) (428) have corroborated. De Pasqualini and Mancini (1951) (429) carried out similar experiments in guinea pigs as well as rats. Their grafts were unsuccessful in the guinea pigs, a fact which, they suggest, indicates that thyrotropin is not increased after thyroidectomy in this species; their grafts were always successful, however, in rats. De Pasqualini and Mancini (1951) (430) found that the pituitaries of their experimental animals had the histologic appearance characteristic of the same glands in incompletely thyroidectomized animals. Such a finding might indicate that some of the graft's normal production of thyroxin was being destroyed but might also mean only that hormone from the intrasplenic graft was not a complete substitute for the full production of the normal gland.

Cordier, Crapa, and Martin (1951) (431) found that there was no growth stimulus to the graft if one lobe of the thyroid remained intact in the neck. On the other hand, if both lobes were put in the spleen, the thyroid tissue became hyperactive. Since similar grafts in the kidney or in the ear were not stimulated, they interpreted their result as showing that there is partial but not complete, destruction of thyroid hormone in the liver. Gabe and Arvy (1947) (432) and Hamolsky and Gerlach (1952) (433) also believed that there is partial alteration of thyroid hormone by passage through the liver. Rupp (1952) (434), on the other hand, believes that enough hormone to maintain growth and to regulate the rate of thyrotropin production escapes inactivation. Lacour, Oberling and Guerin (1952) (435) have found that intrasplenic thyroid grafts, like ovarian grafts, may develop tumors.

### Transplantation of Thyroid in Clinical Practice

Cristiani (1904) (436) showed that healthy autografts of human tissue still retained their normal appearance when examined 15 months later. A graft into a myxedematous patient remained inactive, and yet Cristiani remarks that the patient had grown and that his intelligence developed. Grafts from a hyperplastic thyroid did not survive. Payr (1906) (437) reported experiments which indicated that the spleen was an especially favorable site for transplants and described a case in which a young cretin received thyroid tissue from the mother with great success, improvement lasting for several years. Similar reports of the successful treatment of myxedema have come from von Eiselsberg (1914) (438) and Wittig (1932) (439). Smith (1911) (440), however, discussing a report by Groves and Joll that a thyroidectomized patient was improved clinically after a graft from another patient, pointed out that evidence purporting to support the notion that homografts survive had been in-

correctly quoted and doubted whether homografts could ever survive. Others e.g. Payr (1914) (441) and Portugalov (1929) (442) recognized that homografts might have a temporary effect due to the hormone contained in the grafts, but thought that the effect would not be permanent. Burdette (1952) (443) tried to transplant fetal thyroid tissue to a patient with myxedema but obtained no evidence of a successful graft.

Thyroid tissue can certainly be autotransplanted with ease in human beings, as Cristiani (1904) (430) first showed. Szilagyi and associates (1953) (444) have used thyroid autografts in nine cases of subtotal and total thyroidectomy. Transplants may resume the accumulation of iodine as early as 10 days after operation and their functional activity increases with time, probably due to the actual growth of the graft. Grafts of hyperplastic tissue show a greater growth potential. Swan, Harper and Christensen (1952) (445) autotransplanted thyroid tissue into a 9-year-old girl whose lingual thyroid they had removed. Biopsies of the transplant appeared normal four months after grafting but at this time there was no uptake of radioiodine. Activity of the graft may have been inhibited by the treatment with thyroid hormone that the girl had received. Attempts to transplant stored thyroid tissue failed although successful transplantation of stored rat thyroid glands has been reported (446). Direct vascular anastomosis of a homograft also failed.

### Transplantable Tumors of Thyroid

Purves Griesbach and Kennedy (1951) (447) have shown that tumors develop in rat's thyroid during long term administration of methyl thiouracil. Such tumors can be successfully transplanted into rats with thyroxin deficiency (and therefore with high production of TSH) and sometimes become malignant. The malignant tumors may ultimately become transplantable, even if there is no extra thyrotropin available. It is noteworthy that thyroid tumors often behave like normal tissue as far as their capacity to concentrate iodine and to incorporate iodide into thyroxin is concerned. Wollman, Morris and Green (1951) (448) have tested this capacity in four different thyroid tumors and found wide variations (see also Wollman, 1953 (449). Wollman, Scow and Morris 1953 (450) and Wollman and associates 1953 (451) who showed that tumors were always less active than normal thyroid tissue of the hosts)

Human thyroid tissues have also been transplanted to the anterior chamber of the eye of guinea pigs following the technique of Greene. Whereas most normal tissue or benign tumor material is absorbed relatively promptly, highly malignant tumors may remain viable for a year or more according to Dobyns and Lennon (1951 and 1952) (452 453) who were not able, however to obtain any uptake of radioactive iodine by the grafts.

## PARATHYROID

Once again we find that Cristiani (1903) (454) was early in the field. Parathyroid glands which he transplanted survived in rats for up to 2 years and in cats for up to 5 years. Halsted's first paper was published in 1907 (455). In it he reports attempts to transplant parathyroid tissue into the spleen of dogs, as had been described by Payr for the transplantation of thyroid tissue. He mentions that as many as seven parathyroids were at one time transplanted into the spleen of each of two dogs, but he does not describe what his results were. His later paper (1909) (456) is the one which provides the evidence for the formulation of Halsted's law—'transplants of endocrine organs are normally unsuccessful unless a deficiency greater than one-half is created.' Autotransplants were successful in 61 per cent of dogs if and only if a deficiency greater than 50 per cent was created. Conversely parathyroid tissue in excess of urgent requirements did not survive. A fragment of tissue no more than 0.25 mm. in diameter was sufficient to keep a dog alive (457). Homotransplants always failed even if a deficiency had been established.

Rojas and Manfredi (1908) (458) successfully transplanted parathyroid tissue under the renal capsule in dogs and found that autografts took in 90 per cent and homografts in 30 per cent of cases. Grafts were said to take more readily in parathyroidectomized animals. Survival depended on revascularization beginning within 24 hours. It was complete in 4 days, and 20 days after grafting the tissue had become normal in appearance. They report the laying down of scar tissue centrally and the proliferation of the outer layer of surviving cells which are such a constant feature in histologic descriptions of the changes following the grafting of any endocrine organ. Total reconstruction of the gland occurs by the sixth or seventh month. Swingle and Nicholas (1925) (459) reviewed the literature of

transplantation of the parathyroid and reported successful autografts in cats. Symptoms of parathyroid deficiency returned when the grafts were removed. A series of homografts was also carried out but the grafts did not survive. They believed that successful treatment of tetany in man with homotransplants depended not on permanent survival of the graft but on a temporary effect produced by absorption of hormone contained in the graft. Cristiani and Cristiani (1923) (460) believed that they had evidence that homografts would survive in rats—for as long as one and one-half years—but the host and donor were brothers and may very well have belonged to an inbred strain.

It is usually said that the success or failure of a parathyroid graft cannot be related to the degree of deficiency of the secretion because there is no known trophic principle of the pituitary which can stimulate activity. Dragstedt (1927) (461) points out, however, that partial parathyroidectomy in rats and dogs is followed by hypertrophy *of any remaining fragments of parathyroid tissue.*

Shambaugh (1936) (462) was the first to make a specific test of the validity of Halsted's generalization. He autotransplanted parathyroid tissue in dogs which had either 25, 50, or 100 per cent deficiency of parathyroid tissue. The transplantation of even a single parathyroid, leaving the three other glands intact, was successful, for the graft maintained the life of the animal when the three intact glands were removed at a subsequent operation.

Numerous other workers, *e.g.* Manley and Marine (1916) (395), Goodman (1916) (396), Cordier, Craps and Martin (1930) (431), May (1933 and 1936) (420, 421) and Dempster and Doniach (1955) (367) who were interested primarily in the response of the thyroid to transplantation, have all found satisfactory regeneration of parathyroid tissue that was transplanted at the same time. In Shambaugh and Cutler's (1936) (463) case parathyroid tissue was histologically normal 31 months after autografting in a 61-year-old woman.

A number of attempts have been made to apply parathyroid grafts to clinical practice in the treatment of tetany following total surgical removal of the thyroid and parathyroid. The most satisfactory procedure is to dissect out the parathyroid from the excised tissue and to transplant it unencumbered with thyroid tissue. Cattell (1929) (464) was successful with this form of treatment but had no success with the homotransplant that he attempted. Others have tried to avoid the uncertainty of successful homotransplantation by tissue culturing the prospective donor material before finally transplanting it. Stone, Owings and Gey (1934) (465) report some success with this method, as have Kooreman and Gaillard (1950) (466) and Gaillard (1954) (467) with a modified technique using newborn material for cultivation. They believe that postoperative tetany was controlled especially when the patients were less than 20 years old. More recently work of the same sort by Escamilla and associates (1957) (468) seems less encouraging.

It has been suggested in connection with the clinical effectiveness of homo- or heterografts that their action may depend on a nonspecific stimulus as they disintegrate. Zwerg (1929) (469) found that homoplastic transplants of the parathyroid into normal cats increased the host's gland to twice the normal size.

Parathyroid tissue has been transplanted close *to various bones by Barnicot (1948) (470) and* Chang (1951) (471). There is specific local resorption of bone near the graft, a fact which indicates that parathyroid hormone has a direct osteoclastic effect on bone tissue.

## PANCREAS

The pancreas is a difficult organ to transplant because of the proteolytic effects of the digestive enzymes which the organ also contains, but Ivy and Farrell (1926) (472), Houssay, Lewis and Foglia (1929) (473), Tripodi and Sherwin (1931) (474) and Wang and Grossman (1951) (475) have all autotransplanted the gland successfully. They found that carbohydrate metabolism in such animals is normal and that the usual digestive enzymes can also be secreted by the graft. Simard (1945) (476) considers that, although transplantation destroys the extrinsic nerve supply, the neuro-insular complexes survive and enable the gland to function automatically.

Browning and Resnik (1951) (477) transplanted embryonic or neonatal tissue from mice and showed that the transplants were able to alleviate the symptoms of diabetes that had been induced in the hosts by the intraperitoneal administration of alloxan. Transplants in the anterior chamber of the eye grew and differentiated. Those into the spleen, however, did not survive. Transplants from one strain to another were also unsuccessful. The development of the trans-

plant was not influenced by the presence or absence of normal pancreatic tissue in the host.

Selle (1935) (478) attempted to improve the results of grafting pancreatic tissue in dogs by culturing the grafts in the serum of the future hosts before transplantation, but without success. Murray and Bradley (1935) (479) also cultured tissue from an adenoma of the pancreas before transplanting it into a diabetic individual the grafts had no effect on the diabetes.

## THYMUS

The thymus has no more than a disputed right to be included in a chapter which deals only with endocrine organs. It must be admitted that none of the work outlined below justifies its inclusion in the group On the other hand, it has certainly contributed largely to the study of leukemia.

Dudgeon and Russell (1905) (480) attempted to graft the thymus (of dogs, cats, and rabbits) into the peritoneal cavity of the same species and the same litter but in every instance the graft rapidly degenerated. In 1926 Jaffe (481) autotransplanted the thymus of young guinea pigs and followed the regeneration of the graft for up to 120 days. All except a small peripheral zone degenerated within a few hours, but by the fourth day the reticulum cells were becoming hyperplastic. Regeneration was usually complete by the third week, when newly formed lobules had differentiated into cortical and medullary zones, and Hassall's bodies had reappeared. Lieure and Bonciu (1931) (482) in guinea pigs and Rask Nielsen and Andreasen (1955) (483) in mice, also found that regeneration of autotransplants in young hosts was complete in about three weeks. Richter and Jaffe (1928) (484) autotransplanted the thymus into the bone marrow cavity where the grafts underwent early changes similar to those already described. Regeneration continued more slowly and finally the graft disappeared apparently because it was enclosed in a bony capsule. Jolly and Lieure (1932) (485) confirmed Jaffe's findings in general but believed that any graft tended to disappear after a few months Other successful results have been reported by Romieu and Merland (1933) (486) in the guinea pig, and Foulds (1934) (487) in chickens.

Rask Nielsen and Andreasen (1955) (483) found that intrastrain grafts in mice were successful and described in detail the histologic reconstitution of the grafts. In addition they showed that $F_1$ thymic tissue (CBA × dba) implanted into either parent strain would not grow but degenerated in about a week

Other recent work has been concerned primarily with the relation of the thymus to the development of leukemia. Thymic transplants, for example, appear to increase the incidence of leukemia and restore the normal level of incidence in thymectomized mice [Law (1952) (488) and Kaplan, Brown and Paull (1953) (489)]

## GENERAL CONCLUSIONS

What then are the general conclusions which can be derived from the detailed layout of experimental observations in the preceding pages?

First, one must briefly refer to those fields which have been omitted from consideration. Of these perhaps the largest is the use of transplantation in studying the control of sexual and gonadal development and differentiation. It must suffice to refer readers to Willier's (490) and Witschi's (491) chapters in 'Sex and Internal Secretions' (1939) and to the 'Symposium on Sexual Differentiation in Vertebrates' (1951) (492) Another long-standing endocrinologic problem once much studied by means of transplantation, is that of the antagonisms between hormones of the two sexes. This old work which is coupled especially with the names of Steinach, Sand and Lipschütz revolved around the grafting of the gonads of one sex into the other sex, and was necessary when pure hormones were not available and the only way of studying their effects was by grafting the organs which produced them.

Taking these omissions into account, the value of transplantation as a technique in the furtherance of physiologic gerontologic, or pathologic investigations relating to the endocrine system, has been abundantly demonstrated. The problems of hormone-dependent tumors, the plasticity of hormone production and hypothalamico-hypophyseal relationships are topics which immediately spring to mind as having been especially illuminated.

The method of transplantation was intended to dissociate the organ under study *from its* natural surroundings and in particular *to remove* its normal vascular and neural connections. In this form it supplies one of the essential criteria for judging the claims of any organ to be classified as an endocrine gland. Most endocrinologists would now agree that all the organs at present recognized to be members of the group (except perhaps the posterior pituitary) can be trans-

planted successfully though some (e.g. adrenal medulla) have only been transplanted with difficulty in recent years and others (e.g. the anterior pituitary) with incomplete success unless they are transplanted to a special site.

But the technique of dissociation from the normal environment has been extended in new and fruitful ways to permit the dissection of the function or regeneration of individual components of glands (e.g. the distinction between cortex and medulla of the ovary or of the three zones of the adrenal cortex) and to permit the study of an endocrine organ in the unusual environment provided by a hybrid or by an animal of a different age.

Everyone seems to be agreed at least that much of the grafted tissue is destroyed before revascularization provides an opportunity for the restoration of normal conditions. This fact can be put to good use in some instances (e.g. in dissociating the function of the outer and surviving layer of the adrenal cortex from that of the inner layers which are destroyed) but in most other circumstances it represents a severe restriction of the usefulness of the method. This is particularly true of transplanted ovaries, which, as we have seen, may lose a large proportion of the original population of oöcytes. Unlike the state of affairs in other glands the loss is irreparable: it cannot be made good by proliferation of surviving cells. It is extraordinary to note the speed with which functional activity of a graft may return—e.g. the uptake of iodine by the thyroid while the histologic appearances are still so abnormal.

The study of autografts has, it is reasonable to say, been bedevilled by the dead hand of Halsted's law, a law which is none the better if it is called, as it seems to deserve to be, Cristiani's law. We can now say that autografts of endocrine glands will succeed even when there is no immediate need for their secretions and realize that we have been confused in the past between the taking of a graft and active function of the transplant. The resolution of past confusion may now perhaps help us to recognize a new problem in the differential response to circulating trophic principles of graft and organ in its normal situation.

But, besides being of value in purely endocrinologic research, grafting of endocrine organs seems likely to be of great importance in the study of the immunologic consequences of transplantation. For there is still a wide measure of disagreement about the capacity of endocrine organs to surmount immunologic barriers. It is perhaps unfortunate that so much work has been done on the rat, such a common and convenient laboratory animal but so often of uncertain genetic composition. It does not seem a misinterpretation of all the data that have been presented to say that endocrine homografts will not survive when wide genetic differences are certain. The difficulties arise when differences are narrow and uncertain. It would not be surprising if, under these circumstances, endocrine grafts survived and were at least partially functional for skin grafts can also put up with some genetic disparity even if the host shows its resentment in the form of a niggling reaction. The real question is whether endocrine organs are more acceptable than other organs and, in particular, we must find out the real meaning of the observation that an ovarian graft may survive in a host which destroys a skin graft from the same donor. Any clear demonstration that endocrine organs as a group have fewer or less potent histocompatibility antigens than other components of the body would have obvious and important general implications.

The value of transplanting endocrine organs in clinical practice is, for the present at least, anything but obvious. Where grafts are feasible they are of little value; where they might be of great value they are not feasible. Restricted as one is to the use of autografts, there seem to be few occasions on which such grafts can provide anything which the pure hormones cannot supply more readily and effectively. This is not to deny the future possibilities of long term storage of frozen material coupled with transplantation by vascular anastomosis in certain circumstances where the homograft reaction can be avoided.

The situation in the clinical field is also confused by uncritical reports and the difficulties of any clinical research into the value of particular treatments. The long term benefits often claimed by many workers may represent a nonspecific form of "shock therapy" to the host's endocrine system which is nonetheless effective for being unorthodox. There is room for further investigation in this particular field, as indeed there is wherever one looks.

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# PART IX

# *Organs*

# PART IX

# *Organs*

CHAPTER 13

# Experimental Transplantation of Lung, Heart, Liver, and Spleen

DAVID M HUME

In this chapter consideration will be given to the transplantation of organs other than the kidney and the endocrines, topics which are discussed elsewhere in this volume. Only those transplantation experiments will be discussed in which restoration of the circulation of the transplanted organ was accomplished by anastomosis of the blood vessels of the graft with vessels of the host. Where applicable the fate of autotransplants will be dealt with also in order to establish the technical possibility of successful homotransplantation of a given type of organ.

## TRANSPLANTATION OF LUNG

### Autotransplantation

The main problems involved in autotransplantation of a lung or a pulmonary lobe are a) thrombosis of the veins b) stenosis of the bronchus, c) atelectasis, d) infection, e) slough of the bronchus from ischemia secondary to division of the bronchial arteries and f) hemorrhage when heparin is used to prevent thrombosis. These difficulties have been overcome by several investigators, and autotransplantations have been successfully performed.

Staudacher Bellinazzo and Pulin in 1950 (1) performed excision and reimplantation of the right middle lobe after removing the retrocardiac lobe to facilitate the anastomoses. They found that the pulmonary veins all thrombosed when united by suture techniques. They therefore utilized vitallium Payr tubes to unite the artery and vein and everting sutures to unite the bronchi. The autografts were functionally and histologically good 12 or more days after transplantation In the following year Juvenelle and his coworkers (2) excised and reimplanted the entire lung in dogs and employed suture techniques for the artery the bronchus, and both pulmonary veins. In one case the animal lived and the lung apparently functioned.

Neptune and his coworkers in 1953 (3 4) performed excision and reimplantation of the entire left lung These authors used suture techniques and resected a portion of the auricle with the pulmonary veins attached so that only one venous anastomosis was necessary The lung was washed out with saline solution containing heparin prior to reimplantation. In one dog the autotransplant was functioning normally more than a year after the transplantation. Bronchoscopy revealed no stenosis at the site of bronchial anastomosis x-rays showed good expansion of the lung and bronchospirometry revealed excellent ventilation and oxygen utilization (4)

In 1954 Hughes, Kehne and Fox (5) performed excision and reimplantation of the left lower lobe in 11 dogs. The upper and middle lobes were removed. Suture techniques were used for all anastomoses. In the first 7 cases the pulmonary veins were anastomosed directly Only 1 of this group survived and the lung was atelectatic and congested in this solitary instance In the next 4 cases a portion of the auricle to which the pulmonary veins were attached was resected and utilized for the venous anastomosis. All 4 animals survived. The reimplanted lobe was found to be functional by bronchospirometry and appeared normal histologically Lanari and his as-

sociates (1950) (6) performed autografts of the lung in dogs and were able to achieve good function and normal histologic appearance.

In general, experience with autotransplants seems to indicate that slough of the bronchus as a consequence of bronchial artery division is not an insurmountable problem, although it may be a contributing factor in the development of bronchial stenosis. It appears that anastomosis of an auricular segment is to be preferred to use of the pulmonary veins and that heparin can be used to flush out the lung but is probably better avoided in the postoperative period. The use of antibiotics is very important. Successful transplantation of the lung is technically possible.

### Homotransplantation

In studies of lung homotransplants most authors have considered the end point of graft "survival" to be the death of the experimental animal which, terminally, became listless and weak and refused to eat. For this reason at the time of examination destructive changes in lung homotransplants have usually been more advanced than they are in other transplanted organs. More specific changes often have been obscured by polymorphonuclear infiltration and thrombosis of small veins within the lung substance or even of the pulmonary vein itself.

Staudacher and his associates (1) and Lanari and his associates (6) were the first to describe experiments with lung homotransplantation, both reports appearing in 1950. Staudacher's group transplanted the right lower lobe, using vitallium Payr cannulae for the artery and vein and everting sutures for anastomosis of the bronchus. There was good immediate function of the homotransplants but this was followed in 6 to 8 days by vascular thrombosis, bronchial suture dehiscence, necrosis and break-down of the grafted lobe. Lanari's group, working with dogs, implanted lung homografts which became necrotic in 6 to 10 days after surgery. Bronchopneumonia was the terminal event.

Davis and his coworkers in 1952 (7) performed homotransplantation of the lung in 5 dogs. They survived 20 minutes, 18 hours, 29 hours, 80 hours, and 8 days respectively. One died of tension pneumothorax in 20 minutes, one lung showed necrosis and thrombosis of the superior pulmonary vein in 18 hours, and 3 died of hemorrhage. No significant data with respect to homograft rejection were obtained. The technique employed included ligation of the left middle lobe artery and vein and anastomoses between the host and graft pulmonary arteries, inferior and superior pulmonary veins, and left main stem bronchi.

Hardin, Kittle and Schaffer in 1953 (8) performed lung homotransplantations in 26 dogs. These authors resected a portion of the left auricle with the lung so that only one venous anastomosis was necessary. The lungs were ischemic for 25 to 37 minutes. The transplants survived for 1 to 12 days, and the nature of the changes in the lungs depended on the survival time of the animal. In dogs dying from 1 to 5 days after transplantation the alveoli were seen to be filled with fluid, while dogs dying later had air-filled lungs. After 1 to 5 days there was marked necrosis of the alveolar walls accompanied by polymorphonuclear infiltration. The lung became atelectatic, and lymphocytic infiltration occurred along with the polymorphonuclear leukocytes.

Attempts were made by Hardin and his coworkers to modify the rejection of the homotransplants in 4 dogs by administering 35 to 45 mg. of cortisone per day throughout the survival period. The authors believed that this slightly prolonged survival and prevented the adhesions between the lung and the parietal pleura observed in homografts in animals not so treated. Benadryl given to 3 dogs in doses of 150 to 200 mg per day throughout the postoperative period was ineffective in prolonging survival. In 3 dogs a total body x-ray dose of 400 r was given prior to lung homotransplantation. This failed to prolong the survival, and the dogs died from leukopenia and penumonia. Heparin had an adverse effect on survival since the 3 dogs treated with it died of hemorrhage. The results of these attempts to alter homograft rejection are summarized in table 13.

In three experiments Hardin and his coworkers used litter mate dogs as donor and recipient. It is noteworthy that the survival time of these animals was prolonged for 13 to 30 days.

Hardin and Kittle in 1954 (9) performed splenectomy in 5 dogs at the time of lung homotransplantation. This failed to prolong the survival of the hosts. In 6 other dogs splenectomy was performed 11 to 16 days before the lung homotransplantation was carried out, but this also failed to prolong survival. It should be pointed out that in 5 dogs these authors removed the

host's right and only remaining lung immediately after the homotransplantation of the left lung had been accomplished. Two of these animals survived 6 and 9 days respectively, proving conclusively that the homotransplants were working.

In 1953 Neptune and his associates (3 4) performed lung homotransplants in 25 dogs. In 21 of these no adjuvant therapy was attempted, and the average survival time was 6 days. It was stated that the lungs functioned for about half the period of the animal's survival as determined by x-ray bronchoscopy and auscultation. After this time the animals became listless refused food, and died. The bronchus became compressed distally to the suture line and filled with bloody, necrotic debris. The lung became enlarged, red, solid and friable. Microscopically there was necrosis and the alveoli were filled with red blood cells and polymorphonuclear leukocytes. The peripheral veins ultimately became thrombosed although thrombosis of the auricular appendage occurred in only 2 of the 25 dogs.

To 4 of their dogs these experimenters administered adrenocorticotropic hormone (ACTH), 10 mg b.i.d. following transplantation. There was a definite increase in survival time up to an average of 25 days. One dog was still alive at 42 days, at which time the transplant was removed because of bronchial stenosis. The dog survived this operation The pathologic changes after administration of ACTH were the same as those observed in the dogs not receiving this hormone but were slower to appear It is interesting that one dog untreated with ACTH was still surviving at 27 days when he died of "distemper" (table 13) Prior to transplantation an attempt was made to desensitize 2 of the recipient dogs by injecting portions of the contralateral lung. This failed to prolong survival. It was stated (4) that litter-mate dogs used in some experiments showed no increase in survival time in contrast to the results reported by Hardin and his associates (8) when they used litter mates. There appears to have been an increased survival time when ACTH was administered in the experiments of Neptune's group while Hardin's group found no marked increase in survival time as a consequence of cortisone administration.

Hughes, Kehne and Fox (1954) (6) reported on homografts of the left lower lobe in the dog after removal of the upper and middle lobes. In their early experiences these authors encountered difficulty with hemorrhage after heparin had been injected into the pulmonary artery, and the practice was therefore discontinued. Technically successful homografts were achieved in 3 instances. In 1 dog which lived for 10 days x-ray revealed a clear lung for 5 days. Thrombosis of the artery and vein with gangrene of the lung was found at autopsy The second dog lived for 7 days congestion of the lung started on the second day The third dog lived for 34 days and died of pneumonia. The lung was congested from the third day *on*, and obstruction of the bronchus occurred at the twenty-eighth day Beginning on the day of operation this animal received 300 to 400 mg. of heparin daily for 3 weeks.

Ellis and Richards, in 1951 (10) implanted homografts of the entire left lung in 10 dogs.

TABLE 13

*Homotransplantation of One Lung*

| Method | Dose | No. of Dogs | Survival |
|---|---|---|---|
| | | | *days* |
| (*Hardin Kittle, Schaffer—1953*) | | | |
| Control homografts | — | 10 | 1–12 |
| Cortisone | 35–45 mg /day | 4 | 4–18 |
| Benadryl | 150-200 mg./day | 3 | 5–6 |
| Heparin | — | 3 | 2–7 |
| Total body x ray | 400 r | 3 | 4–8 |
| Litter mate dogs | — | 3 | 13–30 |
| (*Hardin, Kittle—1954*) | | | |
| Splenectomy | — | 11 | 2–9 |
| (*Hardin—1955*) | | | |
| Sensitized c skin | 20 gm × 2 | 7 | 1–4 |
| Control homografts | — | 21 | av 6† |
| (*Neptune and associates—1953*) | | | |
| ACTH | 10 mg b.i.d | 4 | av 25‡ |
| Litter mate dogs | — | — | — |

Neptune *et al* (1953) (11) state that litter mates were used in some experiments without any increase in survival time No details were given

† One transplant was still good after 27 days

‡ One transplant was removed after 42 days

These authors used suture anastomoses and anastomosed the inferior and superior veins instead of the auricle. They administered heparin to prevent venous thrombosis. The average survival time was 6 days; the range was 1 to 14 days. The animals which survived less than 3 days were bothered by coughing and hemoptysis and never responded well. Four animals survived for more than 1 week. They were active for 5 to 6 days and then gradually became lethargic and died suddenly. Auscultation revealed good aeration for 5 to 6 days with absent breath sounds noted a day or two before death. At autopsy there were usually 25 to 30 cc. of serosanguinous fluid in the pleural cavity and there were adhesions of the lung to the pericardium. The vascular anastomoses were patent.

The animals which died early showed air-filled lungs with minimal atelectasis and little inflammation. Those which died later had wet, heavy non-aerated lungs which had enlarged to fill the entire pleural cavity. The cut surface revealed a meaty consistency from hemorrhage and necrosis of the lung parenchyma. An injection of the pulmonary artery at autopsy showed the major vessels to be patent, while the smaller branches failed to fill. Microscopically the animals dying early showed a beginning pneumonia with polymorphonuclear cells in the alveoli. The animals dying later showed loss of architecture, edema, hemorrhage and abscess formation.

TABLE 14

*Sensitization with skin followed by lung transplant*

| Survival of First Skin Graft* | Survival of Second Skin Graft | Interval between Slough and Lung Transplant | Survival of Lung Transplant |
|---|---|---|---|
| *days* | *days* | *days* | *days* |
| 12 | 4 | 7 | 2 |
| 10 | 3 | 6 | 3 |
| 14 | 4 | 7 | 2 |
| 11 | 5 | 5 | 1 |
| 12 | 4 | 7 | 3 |
| 10 | 4 | 6 | 2 |
| 12 | 3 | 4 | 4 |

* Interval between slough of first skin graft and implant of second skin graft was seven days. Hardin (12).

TABLE 15

*Sensitization with lung followed by skin transplant*

| Lung Removed | Interval between Lung Removal and Skin Transplant | Survival of Skin from Original Donor | Survival of Skin from Second Donor |
|---|---|---|---|
| *days* | *days* | *days* | *days* |
| | 6 | 4 | 12 |
| 5 | | 3 | 10 |
| 4 | 5 | - | 11 |
| 3 | 6 | 5 | 1 |
| 2 | | 6 | 11 |

Hardin (12)

*Lanari, Crovatto and Molins* (1950) (11) reported on lung homotransplants in approximately 300 dogs. They performed a series of autografts as well and sacrificed the animals 1 to 7 days after transplantation. On the first day both the auto- and homotransplants showed moderate dilation of the spaces around the bronchioles and arteries. By the second day this dilation had decreased in the autografts but had increased in the homografts. By the third day round cells appeared in the homografts, often in a perivascular location while the autografts appeared normal. By the fourth day the homografts showed an increase in round cells, the beginning of alveolar edema, and necrosis of the alveolar walls. These changes progressed on the fifth and sixth days leading to necrosis and thrombosis of arteries on the sixth day and total destruction of the transplant on the seventh. The animals died of bronchopneumonia. The function of the homograft and the recipient's own lung were tested separately and the homotransplants were shown to function for a time. Arteriograms demonstrated patency of the vessels. One homotransplant survived for 25 days.

Lanari and his coworkers believed that the round cell was important to destruction of the homograft and that the changes which occurred were like anaphylactic pneumonia. These workers also reported that in collaboration with V. Mathov attempts were made to demonstrate precipitins, agglutinin, anaphylactin or complement changes in the blood of the recipient. Completely negative results were obtained.

Hardin in 1956 (12) performed secondary lung transplants in 7 dogs previously sensitized with two sets of skin grafts. Results of this work are summarized in table 14. The average survival time of these animals was 2.5 days compared with 3.5 days for the control. These experiments, like those performed by Dempster with the

kidney suggested that there were common antigens between skin and lung comparable to those between skin and kidney The reverse experiment was also performed the recipient was immunized with a lung transplant and "challenged" with skin. The average survival time for skin from the donor who had supplied the lung was then 3.6 days compared to 11.2 days for skin simultaneously applied from another donor (table 15) There seems little doubt that homograft antigens are individual and not organ specific.

There is thus good agreement among all workers that 1) lung autografts can function over long periods of time despite certain technical difficulties, and 2) lung homografts usually remain functional for only 3 to 5 days after which they are rapidly involved by alveolar wall necrosis, alveolar edema, round-cell infiltration hemorrhage, thrombosis of peripheral vessels, infarction, abscesses, pneumonia, and total destruction by immunizing processes.

It would appear further that 1) the use of ACTH or cortisone may somewhat prolong homograft survival, although neither one alters the final outcome 2) the use of litter-mates as donor and host may also prolong the survival time of homografts 3) Benadryl, heparin, total body x-ray and splenectomy are without effect upon survival 4) a skin grafted prior to transplantation of a lung from the same donor increases the rate of destruction of the lung when it is subsequently transferred 5) as is the case with other transplanted organs no agglutinins, precipitins, anaphylactins, or complement changes can be demonstrated in the recipient animal at this time.

## TRANSPLANTATION OF HEART

### Autotransplantation

Autotransplantation of the heart has never been reported. Removal and replantation of the heart could be accomplished under hypothermia, with a heart-lung machine, or with a third animal to supply temporary perfusion. Even autotransplantation (to another site) could conceivably be achieved as a temporary measure if a heart and lungs were homografted into the thoracic cavity at the same time

### Homotransplantation

Homotransplantation of the canine heart has been carried out by means of five different basic techniques. Four of these are illustrated in figures 191 to 194

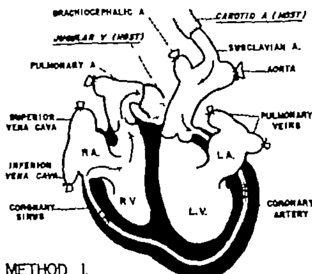



Fig 191

*Method 1*

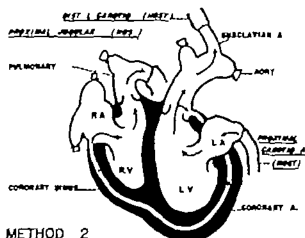
The first technique was devised by Mann and his associates (13) in 1933 (fig 191) Puppy hearts were transplanted to the neck of adult dogs. Coronary perfusion was accomplished by anastomosing the central end of the cut carotid artery of the host to some branch of the transplanted aorta, usually the left subclavian or brachiocephalic. The pressure of the blood against the aortic valves closed them, forcing the blood through the coronary arteries and after perfusion of the myocardium into the coronary sinus. This blood collected in the right ventricle from whence it was removed by anastomosing the pulmonary artery to the central end of the cut external jugular vein. Anastomoses were carried out with the use of Payr cannulas, which permitted the completion of the anastomoses in less than five minutes. The heart thus performed no significant amount of work except that required of the right heart to empty itself of the coronary perfusion blood. The distal cut end of the carotid was anastomosed to the graft aorta by these investigators too a step found to be unnecessary by later workers.

Some workers in using this technique preferred to anastomose only the *distal* cut end of the host carotid to the graft subclavian or brachiocephalic, believing that the decreased pressure and blood flow coming from this end reduced

the likelihood of over-distention of the right heart.

Marcus, Wong and Lusada in 1953 (14) in some cases emptied the right heart by anastomosing the graft vena cava to the central end of the host jugular vein and by creating an intra atrial septal defect to help prevent over-distention of the right heart. This technique is not as satisfactory as use of the pulmonary artery to empty the right heart however. In some experiments these workers also used the femoral and iliac vessels instead of the carotid and jugular and achieved equally good results.

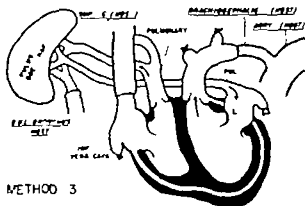
Other experimenters have used some modification of this method (13–19). The method was described by Markowitz in 1040 (20). Marcus, Wong and Luisada in 1952 (18) perfused the coronaries during transplantation by means of carotid and jugular cannulas from a third dog. The carotid cannula was placed in the proximal end of the left subclavian vessel of the graf while the jugular cannula was attached to tl proximal end of the superior vena cava. Th heart and lungs were then removed togethe the lungs being dissected off and the anastomos to the host's jugular and carotid being carri out at leisure while the coronary vessels of tl transplant were perfused by the third dog.




METHOD 2

LEFT SIDE OF HEART WORKING

FIG 102




METHOD 3

HEART PLUS LOBE OF LUNG–
BOTH SIDES WORKING

FIG 103

*Method 2*

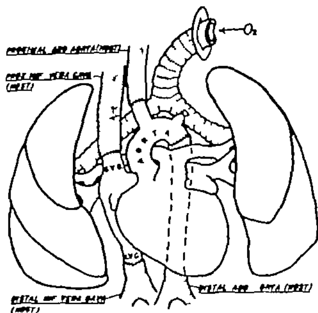
This method (fig. 102) was suggested b Sinitsyn and used by Marcus and associates i 1953 (14) and by Luisada and Marcus in 19 (19). Again the right heart is emptied by ana to mosing the pulmonary artery to the centr end of the jugular vein. This time howeve blood is brought into the left heart by anast mosing the proximal carotid artery to the le atrial appendage. The coronaries are filled b the action of the left ventricle and the exce blood is carried away by anastomosing th brachiocephalic or subclavian vessel to the dista carotid. The left heart performs a significan amount of work, therefore as a result of th technique.

*Method 3*

Matejicek in a brief abstract in 1056 (21) reported the transplantation of the heart an the right upper lobe of the lung into the chest. The method is illustrated as well as possibl from the brief description given in figure 103. The left auricle fills from the left pulmonary vein still attached to it. The coronaries are filled by action of the left ventricle. The transplant aorta is anastomosed to the central or distal end of the host brachiocephalic vessel. The right heart fills through an anastomosis between the superior vena cava of the transplant and the superior vena cava or azygos vein of the host and empties through the right pulmonary artery attached to the grafted lobe and the left pulmonary artery anastomosed to the host superior vena cava or transplant pulmonary vein. An interatrial septal defect is subsequently created. The bronchus of the right upper lobe of the transplant is anastomosed to that of the host after right upper lobectomy. The single lobe is thought to provide sufficient oxygenation for the coronary blood of the transplant and both sides of the heart are working.

*Method 4*

A method used by Marcus and his coworkers in 1953 (14) is useful only for very short term experiments (fig 194) The entire heart and lungs of the puppy are transplanted to the abdominal cavity of the adult dog by anastomosing the vena cava and aorta of the transplant to the inferior vena cava and abdominal aorta of the host. The puppy's lungs are inflated by bringing the trachea to the skin and connecting it to a respirator The recipient thus has two sets of hearts and lungs. Eight animals were subjected to this technique and four survived for periods up to nine hours. The transplant maintained life even after the host heart died—or even when the host lungs were respired only with nitrogen.




METHOD 4 TRANSPLANTATION OF HEART AND BOTH LUNGS

Fig 194

*Method 5*

Neptune and his associates in 1953 (22) succeeded in anastomosing the heart and lungs in their entirety into the thoracic cavity after removing the recipient's heart and lungs. These investigators carried out this procedure in three dogs under hypothermia. The aortic anastomosis was done first, after which the superior venae cavae of the host and transplant were united over a tube. The tracheal anastomosis was next carried out, and the inferior venae cavae of the host and transplant were then united over tubes. Both venae cavae were subsequently joined by suture anastomosis and the tubes were removed. The donor heart was thus responsible for all circulation. The longest survival time was six hours.

**Results of Heart Homotransplantation**

Transplantation of the mammalian heart was first carried out in dogs by Mann and his coworkers in 1933 (13) by means of method 1 The transplantation was accomplished in less than 5 minutes with the use of Payr cannulas. The hearts continued to beat for 1 to 8 days at a rate of 100 to 130 The average survival time was 4 days. The heart rate of the transplant increased when the animal struggled After 24 hours irregularities were apt to develop but this was not invariably so Sometimes the heart would go on beating very well for several days and then stop beating suddenly within 5 minutes At autopsy the surface of the heart was found to be covered with mottled areas of ecchymosis and the heart muscle was friable. Histologically the heart was infiltrated with lymphocytes, large mononuclears and polymorphonuclears. Few normal muscle fibers were present. It was found that this same fate befell the transplant even when a puppy s heart was transplanted into the neck of its mother and it was recognized that some biologic factor was responsible for the failure of the transplants to survive.

Sinitsyn in 1948 (15) transplanted hearts in frogs, cats, rabbits, and dogs. He also used method 1 and his results were comparable to those achieved by Mann and his associates.

In 1952 Marcus, Wong and Luisada (18) reported cardiac transplants in 22 dogs with 10 temporary successes. They used method 1 with three modifications a) the coronaries were perfused during transplantation by the use of a third dog as described above, b) interatrial septal defects were created in some experiments to equalize right and left heart pressures c) the femoral or iliac vessels were sometimes used rather than the carotid and jugular The longest graft survival time was 48 hours.

In 1953 these same workers (14) reported 58 experiments including those reported earlier Interim perfusion was used to cut down the period of anoxia. The animals were separated into three groups. In *group 1* the transplants were carried out by method 1 Temporary success was achieved in 15 cases, and the maximum graft survival time was 48 hours. In *group 2* method 2

was used. There were 22 temporary successes and the longest survival time was 6½ days. In *group 3* method 4 was used. There were 8 technical successes, with survival up to 9 hours. The transplanted heart and lungs maintained life after the animal's own heart beat had ceased. There was no demonstrable increase in survival time among 5 animals of *group 2* which received cortisone.

In 1954 Lubsada and Marcus (19) again reported their accumulated experience. Transplants had been carried out in 101 dogs, with 15 temporary successes with "beating non-functioning" hearts (method 1) and 69 temporary successes with "beating functioning" hearts (method 2). Maximum survival time was still 6½ days. The experiments on the other 32 dogs were preliminary or were operated by method 4. The function of the transplant was studied by observation, palpation, electro- and phonocardiograms, radiography, volume tracings, and angiocardiography. The rhythm was regular and the heart filled and emptied. The injection of epinephrine speeded up the heart. The failure and cessation of the heart occurred over a very short interval. Microscopic examination was made of 4 hearts 3 to 6 days after anastomosis. There was an inflammatory process involving all strata—epicardium to endocardium. This consisted of mononuclear cells, lymphocytes, plasma cells and histiocytes, and was most pronounced in the subendocardial layer. The inflammatory cells tended to collect in perivascular concentrations. There was necrosis of the papillary muscles due to occlusion of small vessels.

Cortisone was administered to 9 animals of *group 2* in an attempt to prolong graft survival. Cross matching of the bloods of donor and recipient prior to transplantation was carried out in 12 cases. In some instances the heart was wrapped in amniotic sac or cellophane. None of these measures prolonged graft survival.

Downie in 1953 (17) transplanted 30 hearts and achieved 23 survivals. He used method 1 employing puppies 6 to 8 weeks old as donors. The average duration of survival was 120 hours; two transplants survived 210 and 245 hours respectively. Histologically the transplants showed infiltration of mononuclear cells, especially in the epicardium. There were areas of degeneration, necrosis and round cell, polymorphonuclear and fibrous tissue proliferation. Unlike the preparations to which Mann referred, however, there were relatively few leukocytes observed.

Wesolowski and Fennessey in 1953 (16) placed transplants in the necks of dogs by method 1. None functioned over a week. The hearts which continued to beat for 4 to 6 days showed foci of softening and hemorrhage in the ventricle with degeneration of the ventricular and atrial myocardium. The hearts which ceased beating after 1 to 2 days showed cellular infiltration chiefly of histocytes, beneath the endocardium. There were depositions of fibrin on the epicardium beneath which there were foci of round cell proliferation. By the third day patchy areas of myocardial necrosis appeared accompanied by diffuse cellular infiltration and edema of the myocardium. The greatest concentration of cells appeared about large and small blood vessels.

Also in 1953 Neptune and his coworkers (22) accomplished complete transplantation of homologous heart and lungs in 3 dogs. These authors used hypothermia and maintained coronary flow during transplantation. Method 5 (above) describes their procedure. The donor heart was responsible for all circulation. The longest survival time was 6 hours. The dog in question showed a return of reflexes, spontaneous respiration, and restoration of body temperature to normal from hypothermic levels. While this method is very interesting as a technical *tour de force*, the resultant survival time is as yet too brief to yield much data pertinent to host immunity. It appears to the present author, however, that this procedure may prove very fruitful if survival can be adequately prolonged, because of the possibility that with the transfer of both heart and lungs, the homologous lungs may act as an antibody trap. All blood perfusing the coronaries must pass through the donor's lung first and thus may be cleared here of antibodies. Under these circumstances one might expect that antibody-induced changes in the heart would be absent or develop more slowly.

The method briefly mentioned by Matejcek in 1956 (21) also lends itself to a test of the antibody trap possibility since the technique consists of the transplantation of the heart and one lobe of the lung together (method 3 above and fig. 103). In some of his experiments Matejcek anastomosed the left pulmonary artery to the left pulmonary vein, or created an interatrial shunt. These procedures would, of course, bring recipient blood directly into the coronaries

without passage through homologous lung tissue and would not serve the experiment postulated. Matejicek carried out his procedure in 21 dogs and noted survival up to 5 days. The recipient s heart and lungs except for the right upper lobe, remain intact and function in this procedure of course. No details of results were reported

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Transplantation of the heart in either a nonfunctioning or functioning capacity is technically feasible but, as is the case with the kidney and lung, fails because of rejection by the host.

## TRANSPLANTATION OF LIVER

### Autotransplantation

No reports on autotransplantation are available. It should be technically feasible, however

### Homotransplantation

Homotransplantation of the canine liver has been carried out by Welch and Goodrich and their coworkers. Welch in 1955 (23) reported that hepatic coma could be controlled for from several hours to a few days by hepatic homografts. His experiments were carried out to determine whether the procedure was technically possible, since the injured liver has remarkable powers of recovery and to determine whether transplanted liver would be tolerated by the host as well as or better than transplanted kidneys. The procedure was somewhat complicated by the necessity of reducing anoxia to the shortest possible time, and by the bacterial invasion present in the canine liver The latter difficulty was overcome by treating the animal pre- and postoperatively with broad spectrum antibiotics. Prolonged anoxia was avoided by creating a temporary shunt during transplantation The liver was transplanted into the lower abdomen and was found to secrete bile and function up to 5 days. Attempts to transplant the liver in monkeys met with failure.

In a second brief note Welch (24) stated that he had performed 47 transplantations and had achieved temporary success in 14 instances The transplants produced bile for 4 days and survived for a minimum of 5 days. At autopsy they were found to have a periportal infiltration of round cells and polymorphonuclear leukocytes with areas of focal necrosis progressing to general necrosis and failure.

Goodrich and his associates in 1956 (25 26) reported the results of these studies in detail. The liver was transplanted to the lower abdomen, while the recipient s own liver remained in place. According to the technique 1) the vena cava of the transplant was united to the proximal vena cava of the host with Blakemore tubes, 2) a temporary polyethylene shunt was created from the aorta to the aorta and hepatic artery of the transplant through the superior mesenteric artery of the transplant, 3) the aortic segment of the transplant was then interposed between the cut ends of the host abdominal aorta by suture anastomosis, and the temporary shunt was removed, and 4) the portal vein of the transplant was connected to the distal vena cava of the recipient over a Blakemore tube

The authors reported 21 successes in 40 attempts (which is a little at odds with Welch s report of 14 successes in 47 attempts) All animals survived for at least 5 days, and all except one produced bile for 1 to 7 days. In 5 experiments the dog's own liver was removed none survived. The secretion of bile was usually maximal 48 to 72 hours postoperatively and proceeded at a rate of 4 to 5 cc per kg Biliary flow usually ceased by the fifth day

Microscopically the first changes noted were small round cell and monocuclear infiltrations of the periportal spaces on the fourth day Thereafter necrosis began and progressed rapidly usually being complete by the end of the fifth day

It would appear from these experiments that the liver too is rapidly destroyed by the immunizing processes of the host in spite of the success attending the technique of its transplantation.

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Histologically the pulp appeared very congested and infiltrated with polymorphonuclear leukocytes In some areas especially at the junction of the pulp and the follicles, there was a deposition of fibrin or fibrinoid necrosis. There were many mitoses of the reticular cells in the germ centers, and the protoplasm of several of the follicular cells had become pyroninophilic but the follicles contained no polymorphonuclear leukocytes. A number of pyroninophilic cells were present in the pulp also The chief features of the microscopic picture were hemorrhage necrosis and leukocyte infiltration in the pulp. The pretransplantation biopsy was normal. The recipient s spleen showed numerous foci of immature plasma cells localized mainly in the pulp with the usual perivascular and peritrabecular concentration.

Simonsen (29) interpreted these changes to mean that the splenic transplant was producing antibodies against the host by means of the proliferating immature plasma cells, just as the host spleen was producing antibodies against the tran plant He pointed out that the immature plasma cells found in the transplanted spleen had the same general distribution as those in the recipient spleen and had probably differentiated from reticular cells of the transplant. The plasma cell were therefore considered to be of donor rather than of host origin.

Samples of the recipient s erythrocytes before and after operation were wa hed three times in saline after which C-S (direct Coombs test) was added The recipient s erythrocytes taken at the start of the experiment were not agglutinated as for those taken 4 days later at the time the splenic transplant was removed agglutination after 5 to 10 minutes at room temperature was grossly visible This positive direct Coombs test was thought to be at lea t consistent with the theory that the transplant formed antibodies against the recipient s individual specific antigens

Eyquem and Oudot in 1953 (30) applied splenic homotransplants to the neck vessels in goats. A swelling of the transplant which occurred in 10 hours diminished slowly and reappeared on the seventh day The swelling did not disappear if there was marked incompatibility between donor and recipient. At the end of the fifth day a positive direct Coombs test appeared, and this effect became maximal on the tenth day In the serum of the recipient an indirect Coombs test could be demonstrated with active antibody in feeble titer against the donor erythrocytes. A puncture of the spleen was carried out on the tenth day The products of splenic necrosis were obtained and centrifuged. The supernatant fluid was found to have antibody by indirect Coombs test. These reactions were obtained with serum antiglobin of sheep absorbed not only with normal sheep erythrocytes, but also with erythrocytes treated with enzyme of RDE type secreted by *Vibrio cholerae* to eliminate interference by anti-T heterospecific agglutinins.

These authors examined the graft microscopically on the fifth day and found cells of a type not present in the recipient s own spleen or bone marrow These were plasmocytes of great density stained basophilic by Unna Pappenheim (pyroninophilic) stain pseudoplasmocytes and histocytes. Erythrophagocytosis was marked, and some animals showed anemia. These results were interpreted as a reaction of the host organs against the spleen, and a reaction of the spleen against the host antigens.

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CHAPTER 14

# Experimental and Clinical Homotransplantation of Kidney

DAVID M HUME

The transplantation of a whole organ by means of surgical reestablishment of the blood supply is qualitatively different in one respect from the homografting of tissue by implantation techniques. In certain cases, such as anterior chamber grafts, the transplanted tissue may be nourished and maintained without developing a blood supply In certain other instances—skin grafts for example—the maintenance of the graft depends on the acquisition of a blood supply from the host. The development and maintenance of such a supply may be adversely influenced by immunologic factors and the graft may be destroyed by ischemia. In the case of organ transplantation on the other hand the grafted tissue has an adequate blood supply from the moment of transplantation, and immunologic alterations leading to graft rejection are mediated through this source The variable introduced by the host-graft interface as it relates to the development of a vascular bed for the graft is absent in organ transplants. Attention can therefore be directed toward changes within the grafted organ itself

Because of its easy accessibility the size and isolation of its blood supply the simple tests for assessing its function and the fact that it is paired the kidney seems an ideal organ to transplant

The first part of this chapter will be devoted to renal transplantation in the experimental animal and the second half to studies in the human being. Only those experiments in which the vessels of the transplant were anastomosed to those of the host to reestablish blood flow through the kidney will be considered.

## Terminology

The terms listed below define in the present discussion the degree of genetic similarity between the donor and host and in some instances identify the transplantation procedure employed. Dempster has suggested (1) that the terms "implantation" or grafting be used to denote the transfers of pieces of tissue or skin which do not effect an immediate blood supply while "transplantation be used to denote whole organ transfer This scheme has not been adopted in this discussion because the terms have been used interchangeably so often in the literature that to redefine them now would lead to confusion

*Autotransplantation.* The transfer of an organ from its original site to some other part of the body with anastomosis of its vascular supply to another artery and vein.

*Replantation.* The removal of an organ and replacement in its original site with reunion to its original vessels.

*Explantation.* The movement of an organ to another site without dividing its vascular pedicle

*Isotransplantation* The transfer of an organ or tissue from one individual to another of identical genetic make-up—such as a monozygotic twin.

*Homoisotransplantation* The transfer of an organ or tissue between genetically similar (closely inbred) although not identical animal

(Permanent "takes" between such pairs have been achieved with tissues, while whole organ transplants have not yet been reported.)

*Primary homotransplantation* The transfer of an organ or tissue from one animal to another of the same species not having close genetic similarity as a consequence of repetitive inbreeding or development from the same ovum. This definition would thus include chimeras and animals with acquired tolerance following *in utero* injections of donor tissue, but would exclude closely inbred strains (homiotransplants) and identical twins (isotransplants)

*Secondary homotransplantation* The transplantation of a second organ or tissue from the donor of a prior (primary) homotransplant.

*Second homotransplantation* The transplantation of a second organ or tissue from a donor other than the original donor of the primary homotransplant.

*Retransplantation*. The removal of the homotransplant from the recipient and replacement in the original donor

*Heterotransplantation*. The transfer of an organ or tissue from one animal to another of a different species.

The experimental studies of renal homotransplantation reported in the literature lend themselves to division into two temporal groups. The first covers that period from 1902 through 1919 when interest was focused on technical details and the proof that autotransplants would function well enough to maintain life while homotransplants would function only for a brief period. The second group covers that period from 1950 to the present time when interest shifted to a more precise definition or redefinition of auto- and homograft function, and particularly to the immunologic mechanisms disposing to homograft rejection. Some of the studies first mentioned in the historic review are applicable to subjects discussed later and are reconsidered in more detail at that time. Some studies after 1949 are included in the earlier group because they are short term physiologic studies not concerned with mechanisms of immunity

### Blood Vessel Union

The early experiments in renal transplantation were a logical outgrowth of interest in blood vessel suture and were usually performed by those contributing to the latter field. Jaboulay and Briau (2) in 1896 described the use of fine interrupted mattress sutures as a means to successful blood vessel anastomosis. Dorrance (3) in 1906 described a continuous mattress suture. Murphy (4) in 1897 described his method of anastomosis whereby the proximal end of the blood vessel was invaginated into the distal end, and held there with sutures. This method was used with some success but failed with renal vessels because their lumens already small, were thus further narrowed and became obstructed by clots.

Payr in 1900 (5) described a non-suture method of uniting blood vessels by means of a prosthesis. This was a short aluminum or magnesium tube through which the blood vessel was threaded. The end of the vessel was then cuffed back over the prosthesis and held in place by a circumferential ligature. The distal end of the vessel was now drawn up over the cuff of the proximal end and held in place by another circumferential ligature. This provided a quick method of intima-to-intima anastomosis but also had the disadvantage of narrowing the lumen. This technique is still used by some workers. A vitallium modification of the Payr cannula designed by Blakemore and Lord (6, 7) and plastic tubes have been used in a similar fashion by other investigators more recently

In 1902 Carrel (8) described his triangulation method of blood vessel suture which made possible an easy end-to-end anastomosis of small vessels and led Carrel and other workers to an extensive investigation of renal transplantation

## I. STUDIES IN EXPERIMENTAL ANIMALS

### HISTORICAL REVIEW (1902–1919)

In many of the early studies of renal homotransplantation in the experimental animal few details of experimental protocol, of urinary excretion and composition, or of pathological findings are given. The period of homograft survival was sometimes measured by the time when urinary excretion ceased and sometimes where bilateral nephrectomy was performed, by the death of the animal. In still other cases the animal was simply sacrificed at an arbitrary time or died of some intercurrent disease and the decision was then made as to whether or not the homograft was still functioning. All of

these factors make it difficult to evaluate much of the early work.

In addition most of the early workers in the field of organ transplantation remained unaware for ten or even twenty years that homograft rejection was due to any factors other than technical failure, thrombosis, infection, or some intercurrent disease of the recipient animal. Although this lapse can certainly be ascribed in part to inaccuracies of observation and procedure in individual experiments, it is still puzzling in view of the repeated demonstrations by many different workers that autografts would continue to function for long periods while homografts would not. However, even the acknowledged titan among the early workers, Alexis Carrel, was unconvinced in 1910 (9) after eight years of work with transplants that renal homografts really behaved differently from autografts, and in 1912, after describing successful skin homografts (10), he criticized those who stated that skin homografts were always rejected. He was at last beginning to accept the concept of homograft rejection by 1914 (11) when he wrote that renal autotransplants were nearly always successful, whereas homografts after an early period of excellent function, "nearly always" were ultimately unsuccessful, and heterografts were always unsuccessful.

Contributing to the failure to differentiate between autograft and homograft function was the intense preoccupation of the early workers with details of operative technique. Attention was focused on 1) the method of vascular anastomosis, 2) the location of the transplant, 3) the handling of the ureter, 4) the control of infection, 5) the perfusion of the kidney, and 6) the comparison of results achieved in different species of animals.

Ullmann in 1902 (12) was the first to carry out renal auto-, homo- and heterotransplantations, using Payr cannulas to make the anastomoses. He performed an autotransplantation first in a pig and then in a dog, using first the inguinal area and then the neck. The single autotransplant reported was anastomosed to the carotid artery and jugular vein, and the ureter was brought to the skin. The transplant secreted urine for five days, after which the ureter retracted beneath the skin and urine continued to come from the wound for a time. No analysis of the urine was made, and no further details were given. In a subsequent very brief note (13) he reported transplanting the kidney from one dog to another and from a dog into a goat. The transplants were placed in the neck. The heterotransplant functioned for a time and was said to have necrotic areas in it at autopsy.

Decastello (14) in 1902 removed a kidney from one dog and placed a homograft in the renal fossa, uniting it to the renal vessels with Payr cannulae. The animal lived for 40 hours and then died of hemorrhage. During the survival period the transplant secreted 1200 cc of urine; it contained albumin and casts but no erythrocytes.

Carrel in 1902 (8), using his new technique of vascular anastomosis, performed several autotransplantations in the neck of dogs. He joined the renal vessels to the carotid artery and jugular vein and brought the ureter to the skin. Carrel noted that the kidneys secreted clear urine, but all transplants were ultimately destroyed by infection, and no analysis of the urine was made. Carrel and Guthrie in 1905 (15, 16) compared the function of a canine renal autograft transferred three days earlier to the function of the animal's normal kidney. They found that the urinary output of the autograft was five times greater than normal and that chloride output was also greater, but that the quantity of organic sulfate and urea excreted was less than in the normal organ.

In 1906 the same authors developed a "patching method" for the anastomosis of small vessels (17–19). According to this method, a piece of the aorta and of the vena cava were left attached to the renal artery and vein, and these were sewn like a patch onto the host aorta and vena cava. Carrel reported this method again in 1907 (20), and he and Guthrie made brief mention of two transplantations made in cats by this technique. In 1906 they also reported an experiment in which they had transplanted two kidneys from one dog to another by an *en masse* technique (21, 22). The kidneys and surrounding tissues were removed with segments of aorta and vena cava of the host. Both kidneys of the host were removed, and the ureters of the transplant were united to the distal ureters of the host. The urine from the transplants was said to be within normal limits on the eighth day. The results of this experiment with a dog which survived for ten days were published two years later (23).

In 1908 Carrel (23) reported the results of transplantation *en masse* of both kidneys after removal of the host kidneys in 11 cat. A piece

of donor bladder containing the two ureters was removed with the transplant. This was sutured to the bladder of the recipient. The kidneys were perfused with Locke's solution before transplantation, and the vessels were united by the suture method. Five animals died in the immediate postoperative period. Seven of the remaining cats died within 14 days. Two animals lived for 31 and 36 days respectively. The first of these animals was noted to have an enlargement of the transplant and marked albuminuria on the eighteenth day. At autopsy vacuolation of the convoluted tubule cells was found, with exudate in the tubules, and these changes were noted to be more marked in the cortex than in the medulla. The glomeruli appeared normal. In the second cat, swelling of the transplant and albuminuria were noted on the sixteenth day. An exploratory laparotomy on the eighteenth day revealed the transplanted kidneys to be edematous and large. At autopsy on the 36th day marked calcification of the aorta and other vessels was noted. The kidneys were said to have secreted urine up to the day of death. A very interesting aspect of this calcification was that it involved the host's vessels but not the aorta or renal artery of the transplant. This finding so impressed Carrel that he wrote two additional papers about it (24, 25).

In 1908 Carrel (26, 27) summarized his results with renal transplants to date and concluded that homografts functioned well until the animal died of infection, hydronephrosis, or some other complication. He felt that the nephritis which he sometimes observed was possibly secondary.

In 1909 Carrel (28, 29) reported a successful renal autotransplant with removal of the other kidney. The progress of the dog so operated was followed-up in 1910 (9) at 23 months and again in 1911 (30) at 2½ years. More will be said about this animal later.

By 1914 Carrel (11) concluded that renal homografts were "nearly always" unsuccessful, that edema of the transplant and round cell infiltration usually occurred, and that cats tolerated homografts better than dogs.

Floresco (31, 32) in 1905 performed renal transplantations to the inguinal area, the neck, and the renal fossa in dogs. He used suture techniques, both Carrel's and Murphy's, and succeeded with one autograft (31). He stated that homografts in the neck lasted from 2 to 6 days, failing because the neck was a poor location; those in the inguinal area lasted only 1 day for the same reason. Those placed in the abdomen lasted from 2 to 5 days if the ureter was brought to the skin and were finally destroyed by ascending infection. If the ureter was anastomosed to that of the host, the kidney functioned for 24 to 72 hours and failed because of stases of blood leading to necrosis. By this interesting bit of inductive reasoning Floresco concluded that the renal fossa was the most suitable location and the inguinal area, the least suitable (32). No analysis of urine was made.

Stich (33) in 1907 worked with renal auto- and homografts in the neck vessels in dogs, bringing the ureter to the skin. The transplants were destroyed by pyelonephritis. Stich then performed one homotransplantation by anastomosing the renal vessels to the iliac vessels and implanting the ureter into the bladder, leaving the normal kidneys in place. The kidney secreted urine which was at first bloody but later clear. The animal died in three weeks with an abdominal wall abscess and peritonitis. The kidney was said to appear normal both macro- and microscopically. No urine collection was made.

In September 1908, at a meeting in Koln, Stich (34) stated that he had performed one autotransplantation of a kidney which proved to be in excellent condition histologically. No details were given. Capelle (35, 36), who was also at the meeting, said he was in the early stages of transplantation work, having observed his transplants only for 0 to 21 days. In one kidney transplanted to the neck, there was a good flow. Capelle did not state whether these were auto- or homografts. Unger (37) was also there and stated that he had done a large series of transplantations. He said that to replace the kidney in its old site was hardly feasible, and that to place it in the neck, while technically easy, led to infection of the wound which prevented long term results. He therefore favored transplantation to the iliac vessels.

Zaaijer (38, 39) in 1908 reported a successful autotransplantation to the inguinal area in a dog in which he anastomosed the renal vessels to the iliac vessels and implanted the ureter in the bladder. He removed the contralateral kidney after 83 days. At the time of this report the transplanted kidney had continued to function for 134 days after transfer. In 1914 Zaaijer reported the final outcome of this case in which the kidney had functioned and maintained the

animal in a state of good health for four years. This was the first convincing demonstration of prolonged function of an autotransplant.

Unger (40–43) in 1909 reported renal homotransplants in 20 dogs and 50 cats He used Carrel's *en masse* technique with suture anastomosis. Most of the transplants did not function, and the animals died of uremia in 2 to 3 days. Significant function was achieved in 3 dogs and 3 cats however The best survival time was 17 days for a dog which remained in good health for 13 days The daily volume of urine was within normal limits during this time and specific gravities as high as 1.039 to 1 052 were recorded The urine showed a trace of albumin and some pus cells and red blood cells, but no sugar or casts. At autopsy the kidneys were enlarged. Microscopically there were numerous bloody necrotic areas The glomeruli appeared normal, and many of the tubules likewise appeared normal The other 5 animals which survived over 3 days are also worth mentioning One dog lived for 5 days and secreted urine for 4 days. At autopsy the kidney was dark red, especially at the border between the medulla and cortex. One recipient was a bitch in the first month of pregnancy The transplant functioned for 9 days, and 2 days later the recipient died. The last 3 animals were cats. One lived for 5 days but produced no urine in the other 2 cases the animals own kidneys were left *in situ* at the time of transplantation. One died in 10 days, and the other whose own kidneys were removed 15 days after the transplantation, died 3 days later

Borst and Enderlen (44) also performed 6 homotransplantations 4 in dogs and 2 in cats. Two dogs and two cats received *en masse* transplants and their own kidneys were left in place, too, giving each of them 4 kidneys. The ureters were implanted in the bladder Three of these animals died in 1 day and 1 died after 18 days, of rupture of the aortic anastomosis. One dog had a single kidney homotransplanted to the splenic vessels. The transplant became necrotic in 14 days. The last dog also received a transplant to the splenic vessels and both of his own kidneys were removed He lived for 2 days.

Unger (45–48) in 1910 mentioned that he previously did *en masse* transplantations in 2 dogs that survived 3 and 4 weeks respectively He then worked with heterotransplants from a pig to a dog from a dog to a goat and 2 from cats to dogs. They were unsuccessful. Unger also performed an *en masse* transplantation of a child's kidneys into an ape, placing the graft vena cava on the host iliac vein and the graft aorta on the host aorta. The ureters with a piece of bladder attached were implanted into the bladder of the host. The ape died 18 hours after the operation was completed.

Villard and Tavernier in 1910 (49 50) and Villard and Perrin in 1913 (51) homografted the kidney in 9 instances in dogs These authors found the *en masse* technique too difficult and had the best results with transplantation to the cervical vessels and next best, with those to the splenic vessels. The longest time any of the transplants secreted urine was 8 days Villard and Tavernier also transplanted a kidney from a dog to a goat without success (52)

Lobenhoffer (53) in 1913 performed renal autotransplants in dogs, anastomosing the renal vessels to the splenic vessels and leaving the ureters intact. The transplants functioned normally

Mantelli (54) in 1913 transplanted the kidneys of a newborn puppy into an adult dog in nine experiments. He anastomosed the aorta and vena cava of the transplant with the artery and vein of the adult. All the transplants failed.

Ingebrigtsen (55) in 1914 performed renal homotransplantation in 2 cats using Carrel's *en masse* technique. One survived and secreted urine for 8 days and was found at autopsy to have thrombosis of the vena cava at the caudal suture site and patent renal vessels and kidneys which were normal both macro- and microscopically The second graft survived 21 days and was technically successful. At autopsy there was no thrombosis infection, or ureteric obstruction. The kidneys showed severe changes. They were enlarged, the boundary between cortex and medulla was blurred, and there were numerous hemorrhages in the cortex. Microscopically the findings resembled an acute nephritis with some lymphocytic infiltration and areas of tubular necrosis. There had been albuminuria while the kidney was secreting Ingebrigtsen realized that the homotransplant failed because of biologic differences between donor and recipient He ran isoagglutinin tests on the cats in these experiments and found no changes accompanying transplant rejection.

Quinby (56, 57) in 1916 performed autotransplantation in 43 dogs of which 10 functioned

successfully for one to three months. The vessels of the transplant were re-anastomosed to the renal vessels, and the ureter was implanted in the bladder. The ability of the autotransplant to maintain life was demonstrated by removal of the contralateral kidney. The animals were followed up for one to three months.

Dederer (58, 59) in 1918 grafted autotransplants in the neck in 6 dogs, one of which was successful. The other kidney of the dog in question was removed two weeks after transplantation, and the animal lived for four months. Dederer showed that the transplant would excrete phenolsulfonphthalein (P.S.P.) rapidly and that the urine flow increased markedly after the other kidney was removed.

Dederer in 1920 (60) performed a homotransplantation of a kidney from one puppy to another of the same litter, making the anastomoses in the neck and leaving both of the recipient's kidneys intact. The animal died of distemper on the twenty-sixth day. The only test of function done was the injection of P.S.P. dye which was said to have been excreted by the transplanted kidney following injection on the twenty-sixth day.

Williamson in 1923 (61) and 1926 (62) using dogs and goats demonstrated that whereas autogenous kidney transplants would maintain the life of the animal for months after the removal of the other kidney, homologous transplants functioned only for a period of days. He described the histologic picture following homotransplantation in the dog as that of acute atypical glomerulonephritis with lymphocytic infiltration, followed by general acute nephritis and ascending infection. If the transplanted kidney was allowed to remain in place until anuria developed, there was a tendency for it to be replaced by fibrous tissue. The author concluded that failure of homologous kidney transplants seemed attributable to a biologic incompatibility between the donor and recipient. He felt that the agent causing this was blood-borne and attacked first the glomeruli and then the tubules.

Avramovici in 1924 (63) reported renal heterotransplantations *en masse* from cat to dog combined with removal of both host kidneys. Two of the heterotransplants were said to have survived for 49 and 58 days, showing normal excretion. At autopsy the transplanted kidneys were said to have been normal. No histologic studies or urinalyses were done. Homotransplantations *en masse* were done in 11 dogs, all of whom had bilateral nephrectomies. One dog was said to have lived for 61 days, finally dying of bronchopneumonia. Another lived for 73 days. No histologic or urinary studies were reported. In 6 additional experiments, bilateral renal homografts were transplanted in two stages. Three of these animals were said to have survived 33, 34 and 58 days. When only one kidney was transplanted, leaving one of the host kidneys *in situ*, survival times of 41, 56 and 60 days were noted. The only mention made of microscopic studies pertained to the animal which survived for 56 days. The transplanted kidney was said to show glomerular lesions, alterations of the tubular epithelium and an inflammation—not very marked—of the interstitial tissues.

Experimental renal hetero- and homotransplantations as successful as those reported by Avramovici have not been achieved by any other worker before or since. Avramovici still believed in 1927 (64) that heterotransplantation was possible with the proper technique and between appropriate species. In view of his omission of microscopic and urinary studies, however, and considering the disparity between his findings and those of all others, one cannot attribute much importance to these results.

Lurz (65) in 1925 performed autotransplantation in 28 dogs. Only two were suitable for studies of function. In these animals the autotransplant was joined to the splenic vessels and the ureter was connected to the bladder. The animals were studied for 10 to 14 days after transplantation, a catheter being placed up the ureter with a cystoscope. Lurz studied function and found that the autotransplant 1) secreted less indigo carmine, 2) had a lower urine specific gravity, 3) had a greater urinary volume and 4) had more chloride output than the normal. Phlorizine diabetes could be induced in the transplant.

Ibuka in 1926 reviewed the literature on renal homotransplantation to that date. He himself did a series of autotransplantations (66) to the neck in dogs to demonstrate that the grafts would function for months. He then worked with 14 homotransplants (67) using the same technique. Two cases were technical failures and 4 succumbed to immediate postoperative sequelae. The remaining 8 cases were carefully followed and the urine analyzed. The transplants

secreted urine for 1 to 5 days averaging 3 days They stopped secreting before infection developed in spite of the presence of an adequate blood flow and the maintenance of a patent ureter Microscopically they showed acute nephritis with lymphocytic and plasma cell infiltration and tubular destruction. The glomeruli appeared fairly normal.

*Holloway 1926 (68) found that homotransplants remained viable for two to four days and that the destructive process started in twenty four hours*

*Brull (69) in 1931 devised a technique for the* study of short term homotransplants in the dog. He utilized three dogs removing both kidneys together with short lengths of aorta and vena cava *from one of them. The kidneys were then* engrafted onto the circulation of the remaining two dogs by attaching the transplant aorta at one end to the carotid of one recipient and at *the other end to the carotid of the second recipient* (called donors by Brull, because they donated blood) The vena caval segment was similarly joined to the two jugular veins Payr cannulas were used *for all connections One of the two re*cipients was then used as the control animal while the other was subjected to various experimental condition The transplanted kidneys *could be perfused by blood from either "recip*ient by applying bull-dog clamps to the aorta and vena cava on one side or the other of the renal vessel By this means the influence of the two recipients on the function of the transplant could be compared Over the past twenty-six years Brull and his colleagues have performed many short term physiologic experiments. These have dealt with phlorizine diabetes (70) urea nitrate nephritis (71 74) permeability to foreign albumin (75, 76) effect of antidiuretic hormone (77 78) the mode of renal action of Parathormone (79 80) comparison of renal function in a transplant removed from an anesthetized dog with that of a transplant removed from a non anesthetized one (81) renal action of thyroxin. (82 87) oxygen utilization (88) anoxia (89) hypertension (90 93) and dietary factors (94 95)

Brull also devised a mechanical heart (96 97) which consisted of a roller pump that massaged the blood through a piece of aorta protected with rubber tubing All connections were made with Payr cannula and were blood vessel lined so that coagulable bl d could be used The blood was then shunted into the carotid-jugular circulation, onto which the kidneys had been grafted, for purposes of studying the effects of various blood pressures on the kidney Both auto- and homotransplants were studied (98) and *in situ* perfusions were carried out as well (99) Brull also reported a technique (100) in which an aortic arch graft was placed as a bridge between the carotid arteries of two recipients and a similar bridge was used to join the jugular veins. Branches of the arch could then be anastomosed to a kidney spleen, or head of a third animal.

*Dor who worked with Brull,* utilized the latter s technique to study oxygen utilization of a kidney removed from an anesthetized dog (101 102) and a non-anesthetized (instantly killed) one *(103) The functional and metabolic recovery* of the kidney after temporary occlu ion of the renal artery was likewise studied in transplants from both anesthetized (104) and non-anes*thetized (105) dogs.*

Compere in 1932 (106) using Brull s method also showed that the polyuria of a hypophysectomized animal was due to a lack of some blood*borne agent having an antidiuretic effect.*

These very interesting experiments deal with renal physiology and are all short term acute experiments performed over a period of a few *hours. Valuable as a source of information on the* functional capacity of auto- and homotransplants in the immediate postoperative period they do not, however illuminate the problems of homotransplant immunity

As a matter of historic record, Frey (107) in 1931 again tried a technique of renal autotransplantation used by Tuffier in 1890 and by others subsequently all without success This consisted of decapsulating the kidney and wrapping the omentum around it to create a collateral circulation. Three months later the renal vessel were divided. Two months later the other kidney was removed The animals all died shortly after this Paunz (108) in 1930 claimed to have achieved success with this method cutting the vessels at two months and removing the unoperated kidney three month later

Herrick Essex, and Baldes (109) in 1932 studied the blood flow of kidneys autotransplanted to the neck for them by Wu Their fin l ings will be discussed *in more detail later*

Wu and Mann in 1934 (110) ma le a microscopic study of the daily progre f auto- and homotransplant in the dog The tran plant

functioned alike until they were removed or the animal died or thrombosis of one or more vessels occurred or pyelonephritis intervened. Some transplants never functioned. This was said not to be due to thrombosis of the vessels. Microscopically monocytic interstitial infiltration and tubular necrosis were observed. The glomeruli still looked quite normal.

Glenn Child and Heuer (111 112) in 1937 and 1938 placed renal autotransplants in the inguinal area in dogs anastomosing the renal vessels to the femoral vessels. The ureter was left intact. The authors demonstrated that constriction of the artery of the transplant led to the production of hypertension. This observation was confirmed and extended by Houssay and Fasciolo (113) in 1938 who showed that the graft of a partially ischemic kidney from a hypertensive dog onto a normal dog whose kidneys were excluded produced a rapid increase in the blood pressure of the normal dog while the graft of a normal kidney did not. Braun-Menendez and Fasciolo (114) in 1940 showed that constriction of the artery of a normal kidney grafted to the neck produced hypertension in two to seven minutes. Blood coming from this partially ischemic kidney also had a hypertensive effect.

Levy Robinson and Blalock (115) in 1938 placed autotransplants in the neck distal to a carotid loop, so that the renal artery blood pressure could be altered in the conscious dog. In three dogs the contralateral kidney was removed after a few days. The renal blood flow and glomerular filtration rate (G.F.R.) decreased as renal blood pressure was decreased by carotid compression. Brull and Dumont (1942) also studied the effect of an ischemic transplant upon blood pressure (90) the stimulating action of uremic blood on the formation by the ischemic kidney of a hypertensive substance (91) and the effect of a normal transplant (92) and an ischemic transplant (93) on the elevated blood pressure of a dog nephrectomized for 48 hours.

Using Payr cannulas Fasciolo and Taquini (118) 1950 performed autotransplantations to the neck in 12 dogs and found that incomplete ischemia of the transplanted kidney produced an increase in renin content even without an intact nerve supply. The effects of transplants on blood pressure will be discussed in more detail later.

Malmejac and his coworkers (117–119) in 1942 autotransplanted the kidney to the neck and then decreased the barometric pressure and measured urine formation. Urine formation began to decrease at 350 mm. of mercury and stopped at 200 mm. of mercury.

Maluf (1943) (120) performed autotransplantations to the femoral vessels, the ureter being placed in the bladder. His results will be discussed later also.

Lefèbvre, in 1946 (121) transferred homotransplants to the neck in five dogs, using Payr cannulas. One failed, two functioned for 24 hours and two functioned for 50 hours. They were able to diurese with water or urea loads.

Parkinson and Woodworth in 1947 (122) performed renal transplantations in goats and referred to them as heterotransplants on the basis that the goats used were of different types. The kidneys were placed in the neck, and anastomoses were made with vitallium tubes. Autografts always developed such good collateral supply that they continued to function even when the vessels thrombosed. Homografts had an active arterial supply and venous return at one week but the kidney had become soft, nearly gelatinous and poorly demarcated. Urine excretion continued for 10 days, but by 14 days the transplant had dissolved.

Oudot in 1948 (123) cooled kidneys to 4°C for periods up to 8 days before transplanting them. Infarction of the kidneys occurred immediately after transplantation. Oudot used Payr tubes of "superpolyamides" as prostheses. Noncooled renal homografts with cutaneous ureterostomies stopped functioning in 10 days. This was felt to be on the basis of a suppurative necrosis due to ascending infection.

Lefèbvre in 1949 (124 125) showed that a homograft can clear urea. He reported that one dog lived for 21 days after homotransplantation with a normal blood urea nitrogen for the first 10 days. The transplants were placed in the neck, and vitallium prostheses were used. The function of the transplant was better if the animal had been bilaterally nephrectomized 24 to 48 hours prior to transplantation (126). This author also studied the effect of perfusing the kidney and storing it at a low temperature before transplantation (127). The transplanted organ was observed only for short periods of time. Lefèbvre stated that kidneys stored up to 24 hours could resume function after transplantation as evidenced by diuresis, chloride output consumption of oxygen, excretion of bilirubin and urochrome and urea output. All of these indices except chloride output

showed the function to be below that of a normal kidney. The maximal urea concentration noted was five times that of blood.

In 1953 Valentino, Florio and Peruzzo (128) reported a technique for anastomosing the renal vessel to the femoral vessels or the neck vessels.

Several references in the literature consist only of general comments on the subject of renal homotransplantation or a summary of the work of other investigators. Articles in this category are those of Carré (1909) (129) who mentioned the work of his assistants Stich and Makkas and briefly discussed Carrel's work, Langlois (1908) (130) who discussed Carrel's work, and Pozzi (1909) (131) who reviewed Carrel's experiments. Pulido Martin (1910) (132) also reviewed Carrel's contributions. A number of other papers on renal transplantation were unavailable to and thus unreviewed by the present writer (133–138).

Several early reviews were compiled. These include a general discussion by Carrel (1905) (139), an extensive review by Stich (1910) (140), reviews by Papin (1908) (141), Morel and Papin (1913) (142), Dahl-Iversen (1929) (143), and a very brief review of some human transplants by Germain in 1951 (144). (More recent reviews will be listed later.)

In the last seven years, as a product of the increased interest in the mechanisms of homograft rejection, a number of studies have appeared on the functional, pathologic and immunologic aspects of renal homotransplants in dogs. Consideration of these in appropriate contexts follows.

## RENAL AUTOTRANSPLANTS

Before a consideration of the results of renal homotransplantation is undertaken, it is important to demonstrate that a renal autograft is capable of normal or nearly normal function.

Floresco (1903) (31) performed one renal autotransplantation that maintained the life of the dog after the other kidney was removed. Zaaijer in 1908 (38, 39) and in 1914 (145) reported the first long term survival of a dog with a single autotransplanted kidney. This animal lived for four years. Between 1909 and 1911 Carrel reported (9, 28, 30) on a successful autotransplant in a dog in which the contralateral kidney had been removed; the animal survived for 2½ years. Borst and Enderlen (1909) (41) reported two autotransplants that maintained the animals for 20 and 110 days respectively. Lobenhoffer (1913) (53) transplanted renal autograft in dogs anastomosing the renal vessels to the splenic vessels and leaving the ureters intact. The transplant functioned normally.

Quinby (1916) (56) performed autotransplantations that supported life for one to three months after which the animals were sacrificed. Dederer (1918–1919) (58, 59) reported one successful autograft that maintained life for four months. Williamson (1926) (62) showed that autotransplants would maintain life for many months. Ibuka (1926) (60) also had long term autograft survivals. Voronoy (1932) (146) worked with 13 autotransplants, two of which functioned for 6 and 15 days respectively; a third one represented a long term survival.

Maluf (1943) (120) autotransplanted the kidney to the femoral region in one dog, explanting the trigone to the skin. He found no evidence of impaired function of the transplant. Nielsen and his associates (147) in 1949 performed four autotransplantations in dogs of which one was successfully functioning at ten months. It was said not to have shown completely normal function. Scott and Bahnson (148) in 1951 autotransplanted eight kidneys using the neck as the site and removing the other kidney. Five of the transplants functioned. One animal died at two weeks; the other four lived one, two, three and four months, ultimately dying with ureteral obstruction and pyelonephritis. Scott and Bahnson also performed five autotransplantations to the iliac vessels, leaving the ureter intact, in animals with experimental coarctation. One survived, the transplant having continued to function for 4½ months.

Nabatoff and his coworkers (149) in 1952 performed 11 canine autotransplantations, three of which were successful. The renal vessels were anastomosed end-to-side to the vena cava and aorta, and the ureter was implanted into the bladder. In the three successful cases, one animal was sacrificed at two months, one at six months, and one was still functioning well at nine months. In 1953 Whelan, McCoord and Schilling (150) performed four autotransplantations to the neck, removing the other kidney and producing experimental ascites. The kidneys were capable of excreting a sodium load.

Of eleven kidneys autotransplanted by Simonsen and his colleagues (1953) (151) only six functioned more than four days and none functioned more than 22 days. The anastomoses were done over Blakemore-Lord tubes, and renal artery

constriction was a major problem. Murray and Holden (1954) (152) reported thirteen autotransplantations to the iliac vessels in dogs with the ureter left intact. Six animals survived for three weeks or more. The transplants were said to be functioning naturally but no details were given. Shimamoto and his coworkers (1954) (153) transferred 15 autotransplants. They were all unsuccessful except for five (of eight) done by Carrel's method of "patching" to the aorta and vena cava. The chief trouble was thrombosis of the renal vein. One kidney functioned for six months and another for three. Of four autotransplantations by Allegra and Basu (1954) (154) one was said to have functioned normally until the animal was killed at 45 days. The ureter was implanted into the bladder.

In 1956 Muirhead and his associates (155) performed renal autotransplantations to the neck in nine dogs. The contralateral kidney was removed either at the same time or 10 to 21 days later. Three animals died early in the postoperative period. The others lived 15, 30, 35 days, 4 months and 12 months respectively, ultimately dying of pyelonephritis. The specific gravity of the urine was 1.010 before the other kidney was removed and rose to a maximum of 1.016. The urine urea was low until the other kidney was removed, and it was then of normal value. Shpuga in 1956 (156) autotransplanted kidneys to the neck in dogs and used a new type of fold-back cannula to assist in the suture anastomosis. He reported an autograft survival of over three years, but the transplant was very atrophied by that time.

In addition to observations on the maintenance of life the early literature reports other functional measurements of the renal autotransplant. *Inulin clearance* was said to be normal by Maluf (120). *P.S.P. excretion* was said to be normal by Maluf (120), Ibuka (66) and Dederer (58, 59). Lurz (65) found that indigo carmine excretion was less than that from the normal kidney. *Renal blood flow* studied by Herrick, Essex and Baldes (109) was found to be normal. The blood flow before sectioning the renal nerves was 142 cc. per min. (measured by thermostromuhr) while after sectioning it was 725 cc. per min. This volume decreased to normal after a period of time. *Responses to antidiuresis and diuresis* were found to be normal by Lobenhoffer (53), Holloway (68), Ibuka (66), Quinby (57), Brull (77, 78), Compere (106), Herrick's group (109) and Shpuga (156).

*Secretion of electrolytes* was studied by Carrel and Guthrie (15, 16) who reported that chloride output was greater than normal. Lurz (65) found the chloride output to be less than that from the normal kidney. *Secretion of sulphate and urea*, was also studied by Carrel and Guthrie (15, 16). They reported that the excretion was less from a transplanted kidney than from a normal one. Muirhead and his associates (155) reported that urea excretion was normal after the animal's second kidney was removed. The *volume of urine* produced by the renal autograft was much greater than the volume put out by a normal kidney according to several investigators—Carrel and Guthrie (15, 16), Lurz (65) and Dederer (59, 60), who noted a marked increase in flow after removal of the animal's other kidney. Maluf (120) found that urine volume was less than normal. Quinby (57) found that it was greater than normal for two weeks, after which it returned to a normal measure. *Specific gravity* was found to be lower than normal by Lurz (65) and by Muirhead's group (155).

These early measures have been repeated recently, and it is pertinent to note the findings in some detail.

Dempster in 1950 (157) and with Watson in 1951 (158) found that kidneys autotransplanted to the neck in dogs did not function as well as the normal kidney, showing as they did a decreased glomerular filtration rate and renal plasma flow. This investigation was extended by Dempster and Joekes (1953) (159) who found a decreased ability of the neck transplant to concentrate and acidify urine and to excrete chloride after saline loading. There was a marked polyuria when the normal kidney was removed. There was also a decline of water diuretic capacity which was regained when the normal kidney was removed. There was slight impairment of electrolyte handling and the authors concluded that the neck kidney was functioning like a hydronephrotic kidney. The blood urea was moderately elevated but the animals were able to live for more than a year with no renal tissue except the neck transplant. Histologic findings up to eight weeks after transplantation were normal.

Dempster and Joekes (160) also studied the diuretic response to an oral water load and to intravenous saline infusions, and the antidiuretic response to the antidiuretic hormone and to subcutaneous faradic stimulation. They found that in the presence of the normal kidney the neck kidney showed a normal diuretic response to a

water load for one to two weeks after transplantation. Between two and four weeks postoperatively however the diuretic response fell to 50 per cent or less than that of the normal kidney and this response remained constant for many months. When the normal kidney was removed, the neck kidney regained a normal diuretic response to water The antidiuretic response of the transplant to antidiuretic hormone or to subcutaneous stimulation, on the other hand, remained normal. The neck kidney secreted urine of low specific gravity from the time of transplantation and this effect persisted even after removal of the normal kidney It was believed that the reductions in renal plasma flow and water diuresis were accounted for by some constriction of the renal artery incident to anastomosis and by scar tissue contraction, although the same authors (159) had earlier attributed these functional defects to some abnormality of ureteric physiology

Murray Lang and Miller (1954) (161) performed renal autotransplantations to the pelvis in dogs, anastomosing the renal vessels to the iliac vessels and removing the other kidney They found that the pelvic transplants were able to concentrate urine normally and that the glomerular filtration rate and renal plasma flow were higher than normal, suggesting compensatory hypertrophy In addition, they found that the transplants did not respond as well as a normal kidney to water loading a finding also contrary to the data of Dempster and his coworkers who found that with removal of the normal kidney the neck kidney showed a normal response to water loading

In 1955 Dempster Joekes and Oeconomos (162) investigated the function of pelvic autotransplants. The renal vessels were joined to the iliac vessels and the ureter was implanted into the bladder The contralateral kidney was removed and the function of the pelvic kidney was therefore compared to previous studies of the single neck kidney and the single normal kidney It was found that there was an initial transient loss of tubular function after pelvic transplantation Within forty days however the transplants showed normal function as judged by the normal specific gravity of the urine, ability to concentrate urea, excretion of a sodium chloride load and maintenance of normal blood urea levels.

Dempster Eggleton and Shuster (1950) (163) studied the glomerular filtration rate of single kidneys transplanted to the iliac vessels and found that, in contrast to the G.F.R. of neck transplants, it was within normal limits

Murray and his associates (1956) (164) studied the function of long term autotransplants in three dogs. The pelvic area was used and the ureter was implanted into the bladder The contralateral kidney was removed and studies of function were undertaken 28 to 38 months after transplantation. Renal function was normal with respect to the specific gravity of urine, diuresis with water loading (in 2 of the 3) P.S.P excretion, glomerular filtration rate, and renal plasma flow The blood urea nitrogen was within normal limits.

It seems that renal autograft function is essentially normal when 1) the kidney is transplanted intra-abdominally 2) when no constriction exists in the anastomoses of the renal vessels and 3) when the ureter is inserted into the bladder so as to prevent ascending infection.

## PRIMARY RENAL HOMOTRANSPLANTS

### Functional and Gross Features

Dempster (1) Simonsen and his associates (151 165 166) and Hume and Egdahl (167) have recently described the functional, gross, and microscopic features of primary renal homotransplants in dogs. The observations made by these workers are very similar and are summarized in the following text.

Dempster (1953) (1 168) described a "toxic syndrome, consisting of high fever and loss of appetite associated with disintegration of the transplanted kidney The syndrome was abolished by antibiotics. Simonsen and his associates (1953) (151) and Hume and Egdahl (167) also noted a fever at the time of homotransplant degeneration. This phenomenon is apparently related to infection and destruction of the transplant.

Dempster in 1953 and 1954 (1 160) described four types of anuria which follow renal transplantation. Two of these were found after autotransplantation as well as after homotransplantation. In type 1 anuria the kidney failed to secrete urine after transplantation. Histologically such kidneys showed cloudy swelling of tubular cells or generalized or local tubule necrosis or widespread cast formation. Dempster believed that this was due to 1) arteriolar spasm perhaps

in part due to nerve stimulation during removal of the kidney or 2) damage to the filtering mechanism incident to the period of anoxia. Simonsen (151) also reported anuria in autotransplants. Type 2 anuria occurred within 24 to 48 hours of transplantation following a period of poor secretion, and was associated with the toxic syndrome. Type 3 anuria was the name given to the cessation of urine flow in primary homotransplants, and type 4 denoted the more rapid decrease of urine elaboration seen with secondary transplants.

It is clear that types 1 and 2 are not related to immunologic mechanisms and are therefore technical problems. Dempster reported (1) that 5 per cent of the autotransplants were anuric from the start while 16 per cent of the homotransplants showed this characteristic. Hume and Egdahl (1955) (167) reported on 41 homotransplants of which three were technical failures, only one showed anuria of a type described by Dempster. Egdahl and Hume (1956) (170) reported on an additional 10 homotransplants none of which showed anuria. No case of technical failure or anuria occurred in the last 30 consecutive transplants done by these workers.

It is possible that the technical success of these authors may be related to three ways in which their technique differs from that of Dempster. 1) Dempster always placed a small bulldog clamp across the bifurcation of the renal artery to prevent saline from entering the kidney when the proximal artery was washed out. This may have contributed to the spasm of the intrarenal vessels there being an intrinsic ability of a blood vessel to go into spasm even in the absence of its nerve supply. Hume and Egdahl allowed the blood to drain from the renal transplant and placed no clamps of any kind on its vessels. 2) Hume and Egdahl made a point of establishing a diuresis in the kidney to be transplanted before it was removed from the donor. The kidney was first carefully isolated so that no connections to the host remained except for the artery, vein and ureter. The prospective donor and host were both given an intravenous infusion of 5 per cent dextrose in water during this time. The ureter was then cut and a small polyethylene catheter was inserted into it and tied in place. The output of urine was observed and when diuresis was adequate the artery and vein were cut and then rapidly anastomosed to the renal artery and vein of the host. The blood supply was usually reestablished within 20 minutes and the transplanted kidney promptly resumed urinary secretion. 3) Hume and Egdahl usually anastomosed the transplant vessels to the renal vessels of the host, while Dempster used either the iliac vessels or the carotid artery and jugular vein. He also often anastomosed the artery end-to-side and the vein end-to-end. It is possible that anastomosing to the renal vessels of the host permits achievement of normal renal vascular dynamics in a higher precentage of cases.

It is interesting that the ability of the kidney to withstand anoxia varies with the species and with the temperature of the animal or transplant. Mitchell and Woodruff (1957) (171) reported that the tolerance of ischemia by sheep kidney was much poorer than tolerance by dog kidney but that the period of tolerance can be extended if the kidney is locally cooled.

Grossly, all transplants showed marked enlargement and edema. There was a progressive reduction of renal blood flow with particularly striking reduction of cortical blood flow (1, 151, 167). Hume and Egdahl (167) examined the cut surface of the transplant and noted several different gross patterns, varying from marked hemorrhage at the corticomedullary junction to edema and focal hemorrhage (figs. 195–198). The diversity of the gross picture was thought to be accounted for by the variations in 1) the rapidity of rejection, which in turn might be related to genetic similarities or dissimilarities, 2) the degree to which each of the microscopic elements of the homotransplant reaction develops relative to the other, and 3) the stage of destruction at which urine elaboration ceases. It is also probable, as suggested below, that differences in the "minor reaction"—namely, that of the transplant against the host's antigens—could account for some of the gross differences observed.

Dempster (1) found that a homologous kidney in the dog survived from 1 to 5 days (average 2.2 days) if transplanted as a third kidney, from 1 to 8 days (average 3.7 days) if one host kidney was removed, and from 1 to 10 days (average 4.4 days) if both host kidneys were removed. The survival was determined by the length of time the kidney secreted urine. Simonsen and his coworkers (151) did not usually measure urine secretion. The bilaterally nephrectomized dogs lived for 5 to 12 days (average 7.8 days). In 7 cases where urine secretion could be estimated it persisted for 5 to 12 days (average 7.8 days). Hume and Egdahl (167, 172) defined the func-

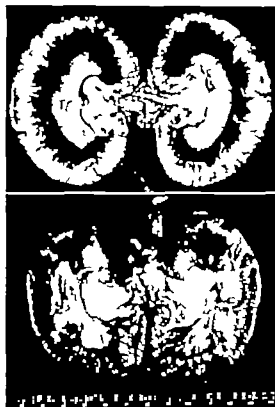


Fig 195 (*above*) The gross appearance of a homotransplanted kidney showing a dark band between the cortex and medulla representing hemorrhage This pattern is quite characteristic

Fig 196 (*below*) Homotransplanted kidney Edema and stippling of the cortex and medulla are present with focal hemorrhage extending into both zones

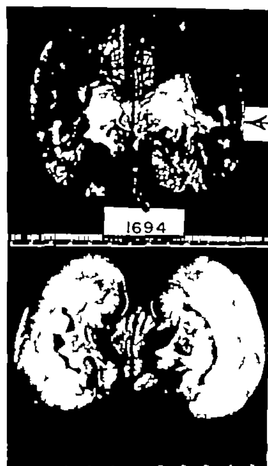



Fig 197 (*above*) Large hemorrhagic bands involve both cortex and medulla. One area of the kidney (see arrow) remained relatively uninvolved

Fig 198 (*below*) Homotransplanted kidney The kidney is large pale and edematous No areas of hemorrhage or necrosis are present

tional period as that time during which the kidney secreted at least 50 cc. of urine in 24 hours, and secretion was deemed to end when the urinary output either fell below this volume or became grossly bloody The transplants in this series functioned for 2 to 14 days (average 5 9 days)

Nielson and his associates (1949) (147) homotransplanted 8 kidneys into dogs and 3 into goats. In 10 of the 11 animals both host kidneys were removed All of the animals died survival times ranged from 2 to 14 days. Baker and his colleagues (1952) (173) performed 5 homotransplantations to the renal vessels. They functioned for an average of 3 ± 1 0 days. Murray and his associates (1953) (174) worked with 9 canine renal homotransplants which functioned for 12 to 96 hours. In 1954 Allegra and Baisi (154) homotransplanted 8 kidneys into dogs. Seven functioned and the animals survived for an average of 4.8 days The transplants had increased in size and there were hemorrhage and edema with granulocytic and histocytic infiltration de Klerk, Scott and Scott (1954) (175) reported on 16 renal homotransplants to the neck vessels in dogs in which the transplant was the only renal tissue present Survival times of 1 to 20 days were recorded. The average time was not given. The glomeruli appeared normal while there were cytoplasmic vacuolation, desquamation, and loss of definition of all tubular elements Archibald and Cawley (1956) (170) reported that 3 homotransplants to the neck functioned for an average of 4.5 days.

Brull and coworkers (1956) (177) tried temporary heterografts from goats to sheep and from cats to dogs. Immediately after the transplantation the blood flow was normal but in a few minutes progressive vasoconstriction developed. These investigators also transplanted simultane-

ous auto- and homografts to the neck in dogs. These were all acute experiments conducted over two to three hours under anesthesia. Venous flows were measured directly. The authors found that at the end of two hours the homografts were excreting 10 per cent less urine than the autografts. There was a progressive vasoconstriction of both kidneys, slightly more in the homografted ones, due partly to anesthesia, increased viscosity of the blood, and, in the case of the homografts perhaps also to an early reaction to the foreign blood.

Humphries (1956) (178) has recently reported his experiences with renal homotransplantation in nine castrated male American Angora goats. The kidneys were placed in the neck and anastomosed to the carotid and jugular vessels, the ureter being brought out through a stab wound. The recipient had been bilaterally nephrectomized 24 hours prior to transplantation. The transplants secreted over 50 cc. a day for 8 to 27 days, with a mean of 15.6 days. The 24-hour output was estimated by collecting urine over a 10-minute to 24-hour period. The calculated output, except for the last 1 to 3 days, was usually more than 500 cc. The specific gravity was as high as 1.021 to 1.030. Weekly N.P.N. values showed a consistent rise. There was a marked increase in kidney size associated with severe edema, and there was very little blood flow through the kidney at the time of sacrifice. Perhaps because of closer relationship between the animals, the transplants in goats survive for a longer period of time on the average than do those in dogs.

The degree of function of a homotransplanted kidney varied but was usually not as good as that of an autograft (1, 151). The homograft was capable of an antidiuretic response (179). Occasionally the function was good for three to four days. When the kidney became anuric it never began secreting again.

## Microscopic Features

Microscopically the homotransplanted kidney in the dog shows striking changes which are always more pronounced in the cortex and which consist of 1) plasma cell proliferation, 2) swelling of the endothelium of the small vessels of the cortex, 3) differentiation of the endothelial cells into plasma cells, and 4) interstitial edema. These changes progress, so that in the later stages there can be noted in addition, 5) avascularity of the glomeruli with spasm of the tuft vessels, 6) focal areas of interstitial hemorrhage, 7) dilation of the tubules and cast formation, and 8) degeneration of the tubular epithelium. If the kidney is allowed to remain in place during the period of disintegration, polymorphonuclear leukocytes appear and secondary infection may occur. These progressive changes are shown in figures 199 to 204.

The homotransplanted kidneys in goats reported by Humphries (178) also showed marked edema and mononuclear cell proliferation in the cortex, with the greatest concentration around blood vessels, especially the vascular pole of the glomeruli. Minimal hemorrhage was present. It is of interest that the goat homotransplants, unlike the dog, showed no endothelial swelling and no evidence of arteritis. This suggests a less violent incompatibility in the goats which may have been due to a closer genetic relationship between them than between mongrel dogs—though perhaps not closer than that between Dempster's greyhounds. This disparity, on the other hand, may have been a species difference, or a consequence of the prior castration of the goats.

In 1953 Dempster (1) and Simonsen and his associates (151) used pyronin stains to demonstrate that the round cell infiltration so characteristic of primary renal homotransplants consisted in fact of plasma cell proliferation. In the early stages the proliferating cells resembled the transitional cells of Fagraeus, while later they appeared to be mature plasma cells. A continuous transition could be seen from elongated, flat, non-pyronin-staining reticulum cells to round pyronin-positive plasma cells. The plasma cell proliferation usually began on the second or third day after transplantation and progressively increased after that. It was accompanied by changes in the endothelium of the interstitial blood vessels. The endothelial cells became very swollen and strongly pyronin-positive. They were cast off into the lumen of the blood vessel, ultimately clogging it completely (fig. 205). It is probable that these cells differentiate into plasma cells and contribute to the marked interstitial infiltration. These changes do not occur in the renal artery itself, or in the vessels of the glomerular tuft.

Simonsen's group studied the histologic picture of transplants removed at three to four days when they were usually still secreting urine, and at about a week during the terminal phase. No correlation between urinary function and histol



FIGS 199-202 Photographs of biopsies taken at representative periods throughout the course of renal homograft rejection in a single animal

FIG 199 (*upper left*) First day Normal

FIG 200 (*upper right*) Fourth day Early interstitial plasma cell proliferation has begun Glomeruli and vessels appear normal

FIG 201 (*lower left*) Seventh day Interstitial plasma cell proliferation has increased Some glomeruli are ischemic There is early tubular degeneration In some vessels very early endothelial changes may be seen

FIG 202 (*lower right*) Tenth day There is advanced plasma cell proliferation and tubular necrosis Almost all vessels show marked endothelial changes and thrombosis

ogy was usually possible because of difficulties in urine collection. Dempster occasionally took biopsies of the transplants, but this was usually avoided for fear of infecting the kidneys. He usually examined the kidneys when they were deemed to be in the process of disintegration. In other instances the kidneys were removed while they were still secreting urine (160)

Hume and Egdahl usually removed the transplants when urine secretion fell to 50 cc. or below in 24 hours or when the urine became grossly bloody whichever occurred first. In a few instances the kidney was removed while it was still secreting well. In addition in four dogs zippers incorporated in the operative wound made it possible to obtain daily biopsies of the transplanted kidney without anesthesia. The progressive changes of rejection could thus be correlated with function of the homograft (167) It was found that 1) there was marked individual difference in the rapidity with which microscopic changes occurred 2) in general urine flow tended to persist longer in those animals in which microscopic changes were slowest to develop 3) the transplant might cease to function however when the microscopic changes seemed less advanced than in another transplant that was still functioning, 4) there was a fairly close correlation between interstitial cellular infiltration and endothelial change in the blood vessels of the cortex, 5) tubular damage appeared to be related to ischemia, and 6) the transplant became progres-



FIG 203 and 204 Photomicrographs of a biopsy taken from a kidney on the day that urine flow ceased

FIG 203 (*above*) One area is shown in which there is mild tubular dilation but almost no edema or plasma cell proliferation

FIG 204 (*below*) Another area of the same slide shows marked edema tubular dilation and degeneration This illustrates the fact that these changes are sometimes very localized This is the same kidney as that shown in figure 198

sively swollen and ischemic as the interstitial infiltration and vascular changes became more pronounced.

Simonsen (1953) (151) and Dempster (1953) (1) presented evidence suggesting that the plasma cell infiltration accompanying renal homograft rejection is not of host origin but arises from cells of the transplant. The evidence for this may

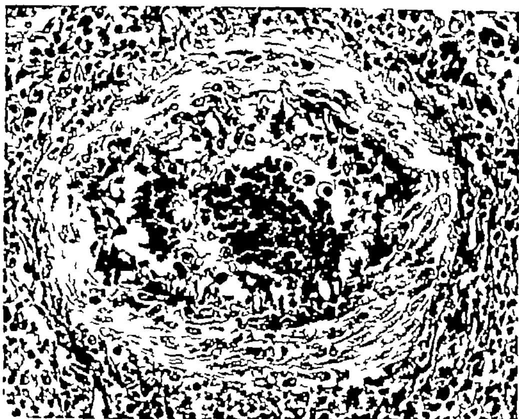


FIG. 205. A photomicrograph of a small artery in the interstitial tissue of the cortex of a homografted kidney. A severe homotransplant reaction is apparent. The endothelium has proliferated and separated from the wall of the vessel, forming a cellular plug. The entire wall of the vessel is involved in a necrotizing process.

be summarized as follows: 1) Plasma cells continue to differentiate after retransplantation of the kidney back into the donor (1, 180, 151). 2) While the transplant is secreting, the plasma cells appear healthy. After the kidney has succumbed to the immune processes, the cells show varying states of disintegration, suggesting that plasma cells suffer the same fate immunologically and at the same time as the rest of the renal cells (180). (It is possible, however, that the disintegration of the plasma cells might be a consequence of the ischemia accompanying homograft destruction, to which all cells, host or graft, might succumb. There are, after all, degenerating polymorphonuclear leukocytes of undoubted host origin.) 3) Much better evidence is the finding by Dempster (181) that when the donor animal was pretreated with cortisone the transplanted kidney was still destroyed but plasma cell infiltration and differentiation of endothelium into plasma cells did not occur. Treating only the host with cortisone did not prevent plasma cell proliferation. 4) Irradiation of the donor kidney 24 hours before transplantation eliminated or markedly reduced plasma cell infiltration without increasing the survival time of the transplant (1). Total body irradiation of the host did not prevent plasma cell infiltration; however, neither did it prevent the destruction of the kidney, which suggests that it was an insufficient amount to suppress antibody formation. 5) By the use of pyronin stains it is possible to demonstrate that differentiation of the intracortical vessel endothelium into plasma cells takes place. In figure 205 a small vessel is shown in which the endothelial proliferation had advanced to the stage where there is complete plugging of the vessel.

Simonsen (151) submitted the hypothesis that plasma cells develop as a response not to host antigen but to host antibody acting as an antigen. The fixation of the host antibody on the endothelial cells of the blood vessels of the transplant provides an antigenic stimulus to those cells to differentiate into plasma cells. Dempster (180) took the view that host antigens provide the antigenic stimulus for proliferation by the transplant of plasma cells from reticular cells and from endothelial cells. He offered two argu-

ments to refute Simonsen's hypothesis 1) Total body irradiation of the host does not prevent the development of transplant plasma cells. (But it also *does not permit survival* of the transplant.) He assumed that irradiated hosts are not producing antibodies and hence that antibodies cannot be responsible for evoking the plasma cell proliferation. 2) Transplant plasma cells are healthy when the transplant is secreting, but disintegrate as the transplant is destroyed (i.e. is overcome by antibody) so that they cannot be evoked by antibody

The first argument is obviously not valid since the irradiated animals are producing enough antibody to destroy the transplant, and may be capable therefore, of producing enough to stimulate plasma cell production. Dempster admits it might be added, that it is the host's reaction against the transplant and not the reverse that is the chief factor in the destruction of the transplant. The second argument is a more cogent one but subject to the objection that the disintegration of the homotransplant is accompanied by marked cortical ischemia which could destroy plasma cells even though they were evoked by antibodies.

What would seem to be a more convincing argument against Simonsen's host antibody antigen theory is a finding cited by Dempster but not in this connection, from observations of secondary transplants in dogs in which the primary transplant was removed before function ceased. These animals might be considered to be partially sensitized by the primary transplant, but not sufficiently to evoke a typical secondary transplant reaction. Surely however the secondary transplant must evoke at least as great an antibody response as a primary transplant. Under these circumstances a rich plasma cell response occurs in the interstitial tissue, but the endothelial cells—upon which host antibody is alleged to fix—do not differentiate into plasma cells, as they do in primary transplants. Furthermore secondary transplants in dogs immunized by a primary transplant which remains in place until it disintegrates are rapidly destroyed, presumably by excess amounts of host antibody often without any appreciable plasma cell reaction.

Dempster (180) cited data suggesting that in 4 of 10 secondary transplants in partially immunized dogs the plasma cell reaction was more pronounced than it was in primary transplants. These secondary transplants were usually not removed until 4 or 5 days after transplantation, however It has been the present author's experience that there is a great deal of individual variation in the amount of plasma cell proliferation at this time in primary transplants some primary transplants at 4 days show tremendously advanced proliferation. This observation therefore, cannot be brought to the support of Simonsen's contention.

Darmady and his associates (1955) (182) performed microdissections of 10 homotransplanted dog kidneys. They found that there was a narrowing of the glomerular tubular neck with epithelial atrophy This was more striking in the outer cortical than in the juxta-medullary zone and was not found in 2 normal or 4 autotransplanted kidneys. This change was partially abolished by cortisone pretreatment in 2 dogs. Secondary transplants showed flattening of the proximal tubular epithelium and dilation of the lumen in addition to the glomerular neck changes. Homotransplants which were returned to the original donor after 48 hours showed only the changes in the glomerular neck. The neck lesions were also seen in secreting kidneys removed 4 to 5 days after transplantation, so that they were not necessarily associated with anuria. A transition from renal reticular cells to plasma cells was described. The suggestion was advanced that the vascular endothelium of the homotransplanted kidney may be the site both of antibody production (against the host) and antibody binding.

In summary it seems that the plasma cells are of transplant origin and that they arise as a consequence of transplant reaction against host antigens, not host antibodies *as postulated by* Simonsen.

## Changes in Renal Blood Flow

Dempster (1953–1955) (1 169 180) has emphasized the role played by cortical ischemia as *an important factor* in the destruction of the homografted kidney He performed nephro-arteriograms by injecting Pyelosil into the renal artery before removing the transplant and found that, whereas in autotransplanted kidneys there was an even distribution of the Pyelosil throughout the cortex very little of the dye entered the cortex of the homotransplanted kidney when it was examined after secretion had ceased (1)

Dempster (1954) (169) further found that 1) arteriograms taken one hour after transplantation

appeared normal. 2) Arteriograms of type 1 anuric kidneys were variable when taken shortly after transplantation but always showed severe arterial spasm with virtual ischemia of the cortex when taken at 48 hours or more. (Type 1 anuria designates the transplanted kidney—auto- or homotransplant—which fails to secrete urine at any time after transplantation.) This vasoconstriction was intensified rather than decreased by Priscol 3) Arteriograms of secondary transplants were normal when taken one hour after transplantation but show generalized spasm or cortical ischemia when taken after the onset of anuria.

Dempster drew several conclusions. 1) The blood flow through anuric kidneys is usually markedly reduced. 2) With cortical ischemia there is an increased renal peripheral resistance. The total renal blood flow is reduced, and what flow there is usually is shunted to the juxta-medullary vessels. In extreme cases even this pathway is closed, and very little blood flows through the kidney at all. 3) Afferent arteriolar spasm is not necessarily related to nervous stimulation—since sometimes it is absent within an hour after transplantation and present at a later date. 4) When cortical ischemia is present there is usually a reduced renal blood flow even without generalized vascular spasm. The reverse is not necessarily so. 5) The onset of anuria at the time of homograft rejection is always associated with generalized vascular spasm. Dempster also described the following patterns of renal blood flow, without stating whether they had been observed in auto- or homotransplants 1) normal renal blood flow and cortical filling 2) reduced renal blood flow with fairly good cortical filling associated either with secretion or anuria 3) severely reduced renal blood flow with good cortical filling and urine formation.

Further studies on renal vascular spasm were done by Dempster in 1955 (180) In this work the kidney was anastomosed in the neck to the carotid artery and jugular vein. Nephrograms were made by injecting 3 cc. of Thorotrast into the carotid artery In addition when the kidneys were to be removed the renal vein was cut and renal blood flow was measured directly after which India ink was injected into the renal artery Dempster's findings may be summarized as follows 1) The renal arteriogram 30 minutes after homotransplantation was normal. 2) In a homograft secreting four days after transplantation, the enlargement of the kidney was accompanied by stretching and straightening of the normally tortuous intrarenal arterial branches. There was no spasm, however and renal blood flow was normal. 3) After the transplant became anuric there were generalized vascular spasm, cortical ischemia and markedly reduced renal blood flow 4) Vascular stretch *per se* was not the cause of the arterial spasm, because marked vascular stretching in a hydronephrotic autotransplant led neither to ischemia nor anuria.

Simonsen and his coworkers (1953) (151) and Egdahl and Hume (1955) (185) also noted that the development of anuria by the homograft was associated with marked impairment of renal blood flow and cortical ischemia.

## SECONDARY RENAL HOMOTRANSPLANTS

### Both Transplants from Same Donor

Simonsen's group (151) performed secondary renal transplantations in four dogs. Neither accurate urinary volumes nor estimates of duration of function were noted. Three of the four animals had homotransplants from another donor at the same time they received the secondary transplants from the original donor thus complicating the immunologic picture. In three cases the secondary grafts were transplanted at the time the primary transplants were removed, and 3, 4 and 5 days respectively after the primary transplantations had been performed. In one case the secondary graft was transplanted 28 days after the primary at a time when the latter had been almost completely resorbed.

In two of the four secondary transplants histologic changes occurred which were entirely different from those observed in any of the primary transfers. There were marked tubular necrosis, interstitial edema, and hemorrhage, with very little interstitial plasma cell proliferation. Fibrinoid degeneration and necrosis of blood vessels occurred without the endothelial proliferation observed in primary transplants Glomeruli often showed fibrinoid necrosis of capillary loops another feature not seen in primary transplants.

The secondary transplant transferred 28 days after the primary one was said to pursue a course indistinguishable from that of a primary transplant. Simonsen concluded that this was because circulating antibodies were no longer present 28 days after primary transplantation. This did not explain the fact, however that another of the

TABLE 16

*Some results of Simonsen's secondary renal homotransplants*

| Exp. No. | Primary Removal | Secondary Removal | Interval between Transplants | Interstitial Plasma Cell Infiltration | | Vascular Endothelial Changes | | Type Reaction of Secondary Transplant |
|---|---|---|---|---|---|---|---|---|
| | | | | Primary | Secondary | Primary | Secondary | |
| | *days* | *days* | *days* | | | | | |
| 15 | 4 | 4 | 4 | + | 0 | + | 0 | Secondary |
| 17 | 3 | 4* | 3 | + | + | + | 0 | Secondary |
| 18 | 5 | 2 | 5 | 0 | + | 0 | | Primary |
| 19 | Not removed | 4† | 28 | ? | + | ? | | Primary |

0 None or almost none
\+ prominent
? Biopsied Vessels thrombosed when removed on ninth day
† Still functioning

secondary transplants which followed only 5 days after its predecessor also showed a reaction typical of a primary transplant. Since tests of function were not adequate to determine when the secondary transplant ceased functioning the graft was arbitrarily removed or biopsied 4 days after it was placed in 3 cases, and 2 days after in one case. All conclusions about the contrast between primary and secondary transplants were based on the histologic picture at this time In 2 of 4 cases the primary transplant was placed in one renal fossa, the secondary, in the other in one case the primary graft was placed in the neck and the secondary one, in the renal fossa and the reverse of this was arranged in still another case (table 16)

Dempster performed three series of secondary renal transplantations. In the first series (1953) (1) 11 secondary kidneys were transplanted 5 to 18 days after the primary ones. The primary transplants functioned for 2 to 11 days (average 4 1 days) and showed gross and histologic features already described of primary transplants. The secondary transplants functioned for 1 to 3 days (average 1.3 days) 8 of the 11 functioning for only 24 hours. The three secondary transplants which functioned for the longest time were in animals whose primary transplants had functioned the longest and in which the interval between the transplantations was the greatest (nos. 5 10 11 table 17) They showed histologic features which were quite different from those of the primary transplants and which consisted of edema, hemorrhage, casts and virtually no cellular reaction. There was massive death of most nephrons with intense glomerular capillary spasm and fibrinoid deposit.

TABLE 17

*Some results of Dempster's first and second series of secondary renal homotransplants*

| Exp. No | Period of Function | | Days between Transplants | Type Reaction of Secondary Transplant |
|---|---|---|---|---|
| | Primary | Secondary | | |
| | *days* | *days* | | |
| 1 | 3 | 1 | 4–6 | Secondary |
| 2 | 2 | 1 | 3–5 | Secondary |
| 3 | 3 | 1 | 4–6 | Secondary |
| 4 | 4 | 1 | 5–7 | Secondary |
| 5 | 6 | 1 5 | 7–9 | Secondary |
| 6 | 2 | 1 | 3–5 | Secondary |
| 7 | 3 | 1 | 4–6 | Secondary |
| 8 | 2 | 1 | 3–5 | Secondary |
| 9 | 3 | 1 | 4–6 | Secondary |
| 10 | 11 | 1 5 | 18 | Secondary |
| 11 | 6 | 3 | 13 | Secondary |
| 1 | 0 | 2 | 5 | Primary |
| 2 | 0* | 1 | 5 | Primary |
| 3 | 0* | 1 | 5 | Primary |
| 4 | 0 | 3 | 5 | Primary |
| 5 | 0 | 3 | 5 | Primary |
| 6 | 0 | 1 | 5 | Primary |

All were anuric after transplantation and were removed on the first postoperative day

In Dempster's second series (1953) (1) 6 secondary kidneys were transplanted into dogs whose primary transplant had been anuric and was removed on the first post-operative day All the secondary transplants in this group showed

TABLE 18

*Some results of Dempster's third series of secondary renal homotransplants*

| Exp No. | Day Primary Removed | Day Secondary Removed | Days Between Transplants | Primary Plasma Cells | Secondary Plasma Cells | Vascular Changes | | Type Reaction of Secondary Transplant |
|---|---|---|---|---|---|---|---|---|
| | | | | | | Primary | Secondary | |
| 1 | 4 | 4*† | 8 | ± | 1+ | + | 0 | Primary |
| 2 | 4 | 5 | 8 | 2+ | 1+ | + | + | Secondary |
| 3 | 4 | 4‡ | 8 | 2+ | ± | + | 0 | Primary |
| 4 | 4 | 5 | 8 | 2+ | ± | + | + | Primary |
| 5 | 4 | 1† | 8 | ± | 2+ | + | + | Primary |
| 6 | 3 | 1¶ | 7 | ± | 3+ | + | 0 | Secondary |
| 7 | 4 | 5† | 28 | 2+ | 3+ | + | + | Primary |
| 8 | 3 | 5† | 13 | ± | 2+ | 0 | + | Primary |
| 9 | 3 | 4§ | 13 | ± | 1+ | 0 | + | Primary |
| 10 | 1 | 5 | 8 | ± | 3+ | 0 | + | Primary |

* Secreting when removed.
† Died suddenly
‡ Removed while secreting because dog was moribund
§ Secreting only 2 days by Hume's criteria.
¶ Secreted only 12 hours

primary reactions and the author concluded that more than a 24-hour period of exposure to a primary transplant was needed in order to elicit the production of antibodies in the host (table 17)

Dempster then performed a third series of 10 secondary transplantations (1955) (180) The primary transplants were removed on the first to the fourth day usually on the fourth and while still secreting They showed histologic characteristics typical of primary transplants. The 10 secondary transplants were placed 8 to 28 days after the primary ones. Seven of them functioned for 4 to 5 days. Only 2 of the group (nos. 2 and 6 table 18) showed secondary transplant reactions histologically and 1 of these functioned for 5 days so that only one transplant showed the previously described explosive secondary response. The contrast between this series and the author's first series is particularly amazing because there is close correspondence in the two groups between the length of time the primary transplant was left in place and interval between primary and secondary transplantations. Yet in one group 11 of 11 transplants showed secondary reactions, and in the other only 1 of 10 (see table 18) The only apparent difference between the two groups is that the primary transplant had *stopped functioning before it was removed* in the first series, and was still functioning when removed in the second. This would imply that the abrupt cessation of function of the primary transplant is an accurate index of a significant acute increase in antibody titer Dempster in attempting to formulate the difference between these two series, misquoted himself in stating that in his first series the secondary transplant was done "not later than four days and in some instances within 24 hours" after the primary transplant had become anuric. Actually the secondary transplantation was done one week after the removal of the primary in two cases and "within three days" in all other cases (I) It is perhaps also important that Dempster usually used crossed pairs, that is the kidney from dog A was placed into B and that of dog B into A. When the secondary transplantations were done therefore, each transplant had already previously been exposed to the prospective host's antigen and antibody As was mentioned above, 2 of Simonsen's 4 secondary transplants showed primary reactions, one being done 28 days after the primary one but the other being performed only 5 days after (the day the primary transplant was removed) There is some suggestion in these figures that 1) the longer the interval between primary and secondary transplant the less likelihood there is that a true secondary reaction will occur 2) the primary transplant has to remain in place long enough to incite antibody formation, and this interval is measured by the cessation of function of the transplant Neither of these suppositions

TABLE 19

*Some results of secondary renal homotransplants by Egdahl and Hume*

| Exp. No. | Days Function | | Days between Trans-plants | Site | | Reaction of Secondary Transplant |
|---|---|---|---|---|---|---|
| | Primary | Secondary | | Primary | Secondary | |
| 1 | 3 | 3 | 37 | RF | N | Primary |
| 2 | 9 | 1 | 14 | RF | N | Secondary |
| 3 | 5 | 1 | 18 | RF | N | Secondary |
| 4 | 7 | 2 | 133 | RF | N | Secondary |
| 5 | 5 | 0 | 29 | RF | N | Secondary |
| 6 | 4 | 3 | 27 | N | RF | Secondary |
| 7 | 3 | 0 | 22 | N | RF | Thrombosis renal artery |
| 8 | 4 | 0 | 23 | N | RF | Thrombosis renal artery |
| 9 | 6 | 2 | 17 | RF | N | Secondary |
| 10 | 10 | 1 | 41 | RF | N | Secondary |
| 11 | 6 | 1 | 14 | RF | N | Secondary |
| 12 | 14 | 5 | 24 | RF | N | Secondary |
| 13 | 3 | 1 | 21 | RF | N | Secondary |
| 14 | 7 | 2+ | 73 | RF | N | Secondary |
| 15 | 7 | 1 | 17 | RF | N | Secondary |

RF = renal fossa.
N = neck.

seems to have been conclusively proven, however

Egdahl and Hume (1955–56) (185 170) performed a series of 15 secondary transplants (table 19) No 'crossed transplants' were used. Of 60 primary transplants 58 functioned for an average time of 5 9 days. Of 15 secondary transplants, 13 functioned for an average time of 1.8 days. The antecedent primary transplants had functioned for an average time of 6.2 days. Two of the 15 secondary transplants developed renal artery thromboses (Nos. 7 and 8) Two others showed thrombosis and destruction of intrarenal vessels but maintained a patent renal artery and vein. This picture was never seen in primary transplants and was believed to be due to an intense immune response. One secondary transplant showed typical primary transplant features (No 1) Ten of the 15 transplants showed typical secondary transplant features, although one functioned as long as 5 days.

Microscopically the principal features of the primary transplant were interstitial plasma cell proliferation, with variable amounts of interstitial edema and hemorrhage, cortical ischemia, tubular necrosis, and necrotizing arteritis of intrarenal blood vessels with endothelial proliferation and obstruction of the lumen with cells and clot. The secondary transplants, by contrast showed very little cellular proliferation, but did show extensive hemorrhage, capillary damage, and tubular necrosis. Whereas Dempster found that the appearance of a protein precipitate in the subcapsular glomerular space was a characteristic microscopic feature of the secondary transplant, this feature was only occasionally seen by Egdahl and Hume. The latter authors believed that the picture of tubular necrosis and interstitial hemorrhage which was so common in the secondary transplants of their study tended to implicate the blood vessels distal to the glomeruli and the tubules as suspected sites of antigen-antibody reaction. Such a reaction resulting in capillary rupture and tubulorrhexis, would seem to explain best the pathologic findings observed.

In the series of Egdahl and Hume a secondary reaction was observed when the secondary transplantation was performed as long as 133 days after the primary one

In one instance, by contrast, a primary reaction occurred when the secondary transplant was done 37 days after the primary It seems likely on the basis of skin graft experiments, that there is a progressive decline in homograft immunity and that after a sufficient interval secondary renal homotransplants will behave like primary ones. It also seems likely

that antibody titer following primary transplantation has not reached its peak until anuria of the primary transplant (as a consequence of the circulatory antibodies) appears. It is possible that when this point is reached the transplant should be removed so that it will not continue to neutralize antibodies with its antigen. These studies certainly serve to emphasize, however, that there is a great deal of individual variation in 1) the rapidity with which antibodies develop to the transplant, 2) the ultimate level of immunity reached, and 3) the length of time for which this immunity persists. As will be demonstrated later, these observations are also pertinent to transplants in man.

### Secondary Transplantation with Skin, Spleen, and Blood as Sensitizing Tissues

In two experiments Simonsen and his associates (1953) (151) used transplants of the *spleen and skin as sensitizing agents before* performing renal transplantation. The spleen was removed on the fourth day and the renal transplantation was accomplished at the same time. A typical secondary reaction followed. In another experiment a renal graft followed seven days after a skin graft. A mild semi-secondary reaction occurred.

Dempster (1953) (184) performed renal transplantations after sensitizing with skin and found that typical secondary reactions occurred. In 5 *of 6 dogs a renal homotransplant following im*munization with two sets of skin grafts functioned for less than 6 hours, and in the other dog it functioned for only 72 hours. It is interesting in respect to duration of immunity that 4 of the 6 kidneys were transplanted 7 days after the second set of skin grafts sloughed. These results are summarized in table 20. The converse experiment was also performed on 4 dogs. Kidneys were transplanted, and after their rejection skin transplants from the original donor and from another donor were simultaneously applied. The *skin grafts from the original donor survived for* 8 days, while those from the other donor survived for 12 days (table 21). The two sets of experiments considered together strongly suggest that common antigens exist in skin and kidney.

TABLE 20

*Some results of Dempster's renal transplants in dogs presensitized with skin grafts*

| Exp. No. | Survival of First Skin Graft | †Interval between First and Second Skin Grafts | Survival of Second Skin Graft | †Interval between Skin and Kidney Grafts | Duration of Renal Secretion |
|---|---|---|---|---|---|
| | *days* | *days* | | *days* | *hour* |
| 1 | 12 | | 4 | 7 | 3 |
| 2 | 12 | | 4 | 7 | 2 |
| 3 | 12 | 4 | 4 | 2 | 4 |
| 4 | 20 | 4 | 6 | | 72 |
| 5 | 12 | 6 | 4 | | 6 |
| 6 | 12 | 8 | 4 | | 5 |

Removal after 24 hours.

† The interval was measured as the time between the slough of one graft and the operative placement of another.

TABLE 21

*Some results of Dempster's skin transplants in dogs presensitized with renal transplants*

| Exp. No. | Duration of Renal Function | Interval between Removal of Kidney and Graft of Skin | Survival of Skin Graft from Original Donor | Survival of Skin Graft from Second Donor |
|---|---|---|---|---|
| | *days* | *days* | *days* | *days* |
| 2 | 18 | 7 | 8 | 12 |
| 3 | 12 | 6 | 8 | 12 |
| 4 | 0 | 4 | 8 | 12 |
| 5 | 5 | 6 | 8 | 12 |

In 4 experiments in 1956 and 1957 Egdahl and Hume (185, 186) transfused 300 cc. of blood from one dog to another prior to renal transplantation. A primary reaction occurred, indicating failure of the transfusion to immunize the host. These investigators then cross-circulated 15 pairs of animals prior to transplantation. It was found that 11 of the 15 transplants showed typical secondary reactions following periods of cross-circulation as short as 5 minutes and with transplantation as long after cross-circulation as 28 days. Two of the transplants performed 9 and 21 days after cross-circulation showed primary reactions. Two of them had thromboses of both artery and vein. Eleven of the 15 transplants functioned for one day or less after transplantation. Cross-circulation was, therefore, an excellent means of immunization.

### Second Renal Homotransplants from a Donor Other than the Original One

Simonsen and his coworkers (151) worked with second transplants from a donor other than

TABLE 22

*Transplants from donors different from the (original) donors of prior transplants*

| Exp. No. | First Transplant | | Second Transplant | | Interval between removal of First and insertion of Second Transplant | Appearance of Second Transplant (from donor other than original one) | Reaction of Secondary Transplant (from Original Donor) |
|---|---|---|---|---|---|---|---|
| | Duration in Host | Duration of Secretion | Duration in Host | Duration of Secretion | | | |
| | | | | Simonsen and associates | | | |
| | *days* | *days* | *days* | *days* | *days* | | |
| 16* | 4 | 4 | 4 | 4 | 0 | Very little reaction | — |
| 17† | 3 | 3 | 9 | 9 | 0 | 4th day very little reaction 9th day primary reaction | Secondary |
| 18† | 5 | ? | 5 | 0 | 0 | Ureter blocked Infiltration on 2nd day hemorrhage on 5th day | Primary |
| 19† | 23 | ? | 4 | 4 | Primary not removed | Primary transplant reaction | Primary |
| 21‡ | 4 | — | 4 | 4 | 0 | Pronounced primary reaction | Secondary |
| | | | | Dempster | | | |
| 1 | + | 7 | § | 5 | + | See text | |
| 2 | + | 4 | § | 1¶ | + | See text | |
| 3 | + | 4 | § | 1 5‖ | + | See text | |
| 4 | + | 3 | § | 1 5¶ | + | See text | |
| 5 | + | 5 | § | 4 | + | See text | |
| 6 | + | 4 | § | 2 | + | See text | |
| 7 | + | 4 | § | 7 | + | See text | |

* Bilaterally nephrectomized at time of first transplantation

† Bilaterally nephrectomized at time of second transplantation and secondary transplant (from original donor) simultaneously implanted

‡ Primary transplant was spleen rather than kidney

§ Presumably removed when secretion stopped

¶ Anuric after 24 hours

‖ Died after 24 hours

\+ Not stated

? Not determined.

— None

the original one in 5 dogs. In the first of these (exp 10, table 22) the primary transplant was removed while still secreting on the fourth day after transplantation. The second transplant continued to secrete, but after 4 days the dog was vomiting and becoming increasingly uremic so that he was sacrificed at this time. (Both the host kidneys had been removed at the time of primary transplantation.) The second transplant *showed less* pronounced histologic changes than any previous transplant.

In the next 4 experiments a secondary transplant from the original donor and another kidney from a second donor were transplanted at the same time. In experiment 17 (table 22) the primary graft was removed after 3 days and the second transplant showed a typical primary reaction when it was removed 9 days after transplantation. The secondary transplant from the original donor showed a modified secondary reaction at biopsy on the fourth day. In experiment 18 the primary transplant was removed after 5 days. The animal died 5 days later. The transplant from the second donor was biopsied on the second day and found to be hydronephrotic due to obstruction of the ureter. Both the transplant from the second donor and the secondary transplant showed primary reactions. In ex

periment 10 the primary grafts remained in place and the second-stage transplants were placed 28 days after the initial surgery Both transplants showed primary reactions In experiment 21 the *spleen was used as the primary transplant.* The secondary renal transplant in this case showed a secondary reaction the renal transplant from the second donor in this case showed a primary reaction.

Of the five studies experiment 10 may be discounted because the primary transplant was *removed after only four days while still secreting,* and the antibody titer may have been too low to produce a secondary reaction at this time. Experiments 18 and 19 may also be discounted, because even the secondary transplant from the original donor failed to show a secondary reaction. This effect in experiment 19 may have *been due to the relatively long interval* (28 days) between primary and secondary grafting or due perhaps to the fact that the primary transplant remained in place and was continuing to absorb antibodies. Experiments 17 and 21 appear to demonstrate that 1) antibodies were formed against the original donor as indicated by *the typical secondary reaction of the secondary* renal transplant and 2) these antibodies were individual specific as suggested by the presence of a primary reaction in renal transplants from second or different donors. Experiment 17 incidentally suggests that a significant antibody titer against the primary donor had been reached in only *three days and while the primary transplant was still secreting*

Dempster (1) worked with second transplants from new donors in 7 dogs (table 22) In two of these experiments the second transplant became anuric in 24 hours and in an additional case it became anuric in 48 hours These *transplants were said not to show a secondary reaction* histologically In a fourth experiment the dog died in 24 hours. In three experiments the second transplant secreted 4 5, and 7 days (their corresponding primary ones had secreted 5, 7 and 4 days respectively) It was stated that these three transplants did not show the *features of secondary transplants from* the original donor

In some recent experiments Gordon and Richards (1957) (187) attempted to prolong life in 4 bilaterally nephrectomized dogs with successive renal homotransplants from different donors One dog received 4 such transplants, another received 3, and 2 others received 2. The life of one dog was maintained for 28 days. The period of survival of the successive homotransplants *from different donors was not different from* that of the first transplant.

These experiments although rather few in number indicate that a second transplant from a donor other than the original one behaves like a primary transplant.

## RETRANSPLANTATION

A few retransplantation experiments have been done in which the homotransplant has been removed from the recipient and replaced in the original donor Simonsen and his coworkers (1953) (151) performed 5 such experiments (table 23) One of these was a technical failure (exp. 17) Of the remaining 4 transplants, 2 (exp. 14 and 15) had been left in the recipient for 4 days before retransplantation, and showed rather marked histologic changes at the time of retransplantation. The changes in these 2 cases progressed during the seven and four days after their return to the original donor The kidney in *experiment 18* showed very little evidence of interstitial reaction after 5 days of homotransplantation. It was retransplanted at this time and upon removal 2 days later it still showed almost no interstitial infiltration. The final transplant (exp 20) was actually a secondary transplantation. The primary graft had been left in the recipient *for 4 days and showed typical histologic* changes. The secondary transplant was left in the recipient for one hour and was then retransplanted to the original donor as the sole kidney It functioned well for 10 days after which time it was biopsied Following the biopsy the artery thrombosed and it was not functioning when *removed at 13 days.* The histologic appearance at 10 days was normal.

In another series of experiments Simonsen (1955) (189) performed 4 retransplantations of dog kidneys which had remained for one hour in an immunized host. In every case the kidney was necrotic when examined 4 days later This series is at odds with the secondary transplant in Simonsen's first experiments The discrepancy may be related to the method of immunization, which had been by a previous transplant in the first case and by *the intravenous injection of A+* donor blood into an A− recipient in the second.

TABLE 23

*Retransplantation experiments by Simonsen and Dempster*

| Exp. No. | Duration in Host | Time of Removal after Retransplantation | Evaluation of Function | Appearance |
|---|---|---|---|---|
| Simonsen and associates | | | | |
| | | *days* | | |
| 14 | 4 days* | 7 | Poor | Marked primary reaction |
| 15 | 4 days* | 4 | Poor | Marked primary reaction |
| 17 | 3 days† | 6 | None | Ischemia and necrosis |
| 18 | 5 days | 2 | | Dilation of tubules congestion of capillaries almost no interstitial infiltration |
| 20 | 1 hour‡ | 13 | Good for 10 days | Normal 10 days after retransplantation thrombosed artery before removal at 13 days |
| Dempster | | | | |
| 1 | 48 hours | 7 | Stopped suddenly | Severe tubulorrhexis and cellular infiltration mainly of mature and immature plasma cells |
| 2 | 48 hours | 4 | Stopped suddenly | |
| 3 | 24 hours | 8 | Good | Plasma cells and positive vascular endothelial pyronin reaction otherwise normal architecture |
| 4 | 24 hours | 5 | Good | |
| 5 | 24 hours§ | 6 | Good | |

* Rather marked histologic changes at time of retransplantation

† Died Artery partly thrombosed leading to ischemia and necrosis

‡ A secondary transplant the primary one had been in place 4 days

§ Removed because arterial suture line broke down

Dempster (1955) (180) performed retransplantation in 5 dogs (table 23) In two (exp 1 and 2) the transplants remained in the host for 48 hours at which time they were retransplanted to the original donors where they remained for 7 and 4 days respectively *Both of the transplants* functioned for a time after retransplantation and then suddenly ceased When examined at this time they showed severe tubulorrhexis and a cellular infiltration consisting mainly of mature and immature plasma cells. The other 3 transplants (exp 3, 4 and 5) were left in the host only 24 hours before retransplantation. They continued to function well after retransplantation until removed after 8 5 and 6 days respectively Microscopically there was some plasma cell infiltration and pyronin reaction of the vascular endothelium but these were not so marked as they were in the first two cases. *The architecture* was otherwise normal. Dempster concluded from this that one day in a foreign host is sufficient time to evoke the plasma cell infiltration which however does not advance and appears to have no detrimental effect on the kidney A period of two days in a foreign host, on the other hand produces some change which leads to anuria a few days after retransplantation

From Simonsen's experiments it seems that 1) If the transplant remains as long as 4 days in the host *and* has an advanced cellular infiltration at the time of retransplantation it will show a progression of changes after retransplantation and fail to recover It should be noted, however that only 4 days in one case and 7 in the other were allowed for recovery It is possible that recovery from the ischemic changes brought about by homotransplantation may occur eventually after retransplantation, just as changes in *ordinary ischemic nephrosis* are repaired in time. 2) There is a hint (in exp 18) that even after 5

days in the host if cellular infiltration is not already advanced the kidney may remain anatomically fairly normal after retransplantation Unfortunately the kidney in question was removed only 2 days after its return to the original donor and no studies of function were done. 3) A secondary transplant can endure the host for at least an hour without suffering any lasting damage This period of time, however is enough to doom a retransplant which was exposed to the circulation of a host previously immunized by injections of blood from the donor

From Dempster's experiments with 5 dogs it may be concluded that 1) The transplant can endure for 24 hours in the host. After this time processes are under way which lead inexorably to tubulorrhexis, plasma cell infiltration anuria, and failure And yet this conclusion raises questions. It is in contradiction to the results of Simonsen's retransplant (exp 18) which showed no such changes after 5 days in the host and 2 days back in the donor (a total of 7 days) Why then, should Dempster's retransplant (exp. 2) after 2 days in the host and 4 days in the original donor (a total of 6 days) show such severe and fatal changes? Dempster's own belief that 48 hours in the host is long enough to initiate irreversible change begs the question. Dempster apparently did not biopsy the transplants at the time of retransplantation as Simonsen did and therefore one cannot know how far advanced the plasma cell changes were at that time As Hume and Egdahl in 1955 (167) have shown there is a great deal of variation in the degree of plasma cell proliferation present in different transplants at a given time in the post transplantation period. It seems likely that the important factor is how far the proliferative changes have advanced at the time of retransplantation and not how many hours have passed 2) The observation may be valid however that after a certain period in the host, the transplant will continue to function for a few days after retransplantation and then suddenly become anuric and show advanced tubulorrhexis and plasma cell proliferation 3) Dempster (1953) (1 151) showed that pretreatment of the donor with x-rays or cortisone prevented the plasma cell proliferation and endothelial changes usually seen in primary homotransplants without prolonging the life of the transplant. Therefore these changes which constitute the reaction of the graft against the host are not responsible for homograft rejection It may be concluded, therefore, that (a) host antigen-binding to the transplant occurs and leads to progressive plasma cell proliferation after retransplantation, and (b) host antibody-binding also occurs and leads to progressive destruction of the transplant after retransplantation. It would be very interesting to pretreat the donor with cortisone and retransplant into the donor after 48 hours or more in the host to see whether antibody-binding can be reduced—by this means—as continual function of the homotransplant would demonstrate

It would be illuminating to perform additional retransplantation experiments to establish more accurately the critical period or critical event which prevents recovery following retransplantation, and also to compare findings in primary and secondary homotransplantation and after other methods of immunization

## ADDITIONAL IMMUNOLOGIC STUDIES

Various investigators have carried out immunologic studies of renal homotransplants in animals in addition to those described above under secondary homotransplants and retransplants. Efforts have been made to shed light on the mechanism of homograft destruction, the development of immunity methods of modifying immunity processes and so on These investigations will be considered briefly in the following paragraphs.

### Attempts to Demonstrate Circulatory Antibodies

#### *Serum Complement*

Complement fixation tests and complement levels have been determined on the recipient's serum after transplantation to see whether this system of immunity was incriminated in transplant rejection The expectation was that if it were complement fixation should coincide with the development of the antitran plant antibodies, thus decreasing the level of complement in the blood. The reverse proved to be so, however the complement levels increasing after transplantation—apparently as a non-specific response to operative trauma.

Voronoy (140) in 1932 studied complement levels after autotransplantation and found that the complement level was depressed if the transplant failed and the wound was infected but if the postoperative course was smooth there was no change in the complement level Simonsen in

1953 and 1955 (188 189) found no increase in complement fixation following renal homotransplantation in dogs. There was a rise in complement levels 3 or 4 days posttransplantation at a time when incompatibility generally began to manifest itself histologically The author concluded that this was an unspecific operative response and that complement played no part in transplantation immunity In 1953 Favour and his associates (100) and Murray and his associates (174), in two reports on the same experiments, stated that no significant depression in complement levels occurred after transplantation and noted instead a slight non-specific rise.

*Red Blood Cell Agglutination*

Ingebrigtsen (1914) (65) ran isoagglutination tests on cats with renal homotransplants and found no change after transplantation.

Simonsen (1953) (188) performed experiments in which serum from the recipient was mixed with the donor's blood cells in order to determine whether agglutination would occur No agglutination was observed except in one case in which a crossed transplant had been done. Simonsen then attempted to demonstrate incomplete antibodies following transplantation by agglutination in bovine serum albumin or indirect Coombs test. He was able to demonstrate antibody formation against the donor in five recipients. A direct Coombs' test was performed in four recipients to see whether the host's erythrocytes had become sensitized after transplantation as a consequence of antibody formation by the transplant against the host. A positive direct Coombs test was found in one case following transplantation of the spleen, and this persisted even after washing the blood cells three times. In the three cases in which kidney homotransplantation had been done agglutination was also found, but it was negative or doubtful after washing of the cells. These results might be explained on the basis of a better antibody formation by the transplanted spleen than by the less well developed reticuloendothelial system of the transplanted kidney There is no proof of course that transplant antibody formation was responsible for this phenomenon. In one single case following homotransplantation the recipient s blood cells became hyperagglutinable in all dog serum including the recipient s own. The reason for this is not clear

In 1955 Simonsen (189) immunized an A− canine recipient by intravenous injections of blood or washed erythrocytes free of leukocytes from an A+ donor Good titers of anti-A were obtained. Two to 10 days after the last injection a kidney was transplanted from the blood donor In 2 cases the kidney disintegrated more rapidly than normal, being destroyed in 12 hours. The blood passing through the kidney was tested for fall in anti-A titer to see whether this antibody was the cause of the kidney destruction. No fall could be demonstrated Simonsen then retransplanted 4 kidneys into their original donors after each had remained in the immunized host for one hour The kidneys were found to be necrotic 4 days later—a condition not found after one hour or even after 3 to 4 days in a non-immunized host. (See *Retransplantation* above.)

Simonsen also washed out one kidney from an A+ donor homogenized it, and injected it subcutaneously into an A− animal twice a week In 1 of 3 cases anti-A antibodies were formed. An accelerated destruction occurred when a renal transplant was placed in this dog from the donor of the kidney homogenate. In another case in which anti-A antibodies failed to develop a primary reaction occurred and the transplant secreted for 8 days. These very important experiments suggest that there may be some relationship between antitransplant antibodies and anti-A antibodies

Murhead and Groves (1955) (191) reported positive direct Coombs' tests, anemia and increased red cell fragility in dogs receiving homologous renal transplants. The direct Coombs' test was positive after 5 to 10 days. The whole triad abated after 14 to 28 days. The authors did not state whether or not they flushed out the transplants. They believed that these results might be due to 1) non-immune globulin derived from the resorbing homograft which attached to host red blood cells 2) or immune globulin which attached to the red blood cells and acted as an auto-immune RBC antibody or 3) direct RBC injury from products of absorption.

As was mentioned in the chapter on splenic transplants Eyquem and Oudot demonstrated that following homotransplantation of the spleen in goats a direct positive Coombs test developed in the recipient together with a strong agglutination against recipient cells in extracts of transplanted spleens. Simonsen and Eyquem replaced the left kidney in A+ dogs with spleens of A− dogs One week later the host developed a positive direct Coombs test, serum auto-agglutinins,

and a severe anemia all coinciding with disintegration of the splenic graft.

It appears that something in the disintegrating homograft may lead to RBC destruction and anemia. Although matching blood groups of recipient and donor does not seem to be important to the survival of skin grafts, a proper match of ABO types appears to influence the survival of human renal homotransplants as will be discussed later.

### X-ray Treatment of Recipient and/or Transplant

Dempster (1953) (1) irradiated the kidneys of six donor dogs 24 hours prior to transplantation. The dose of irradiation and the method used were not stated. The period of function following transplantation varied between two and nine days (average 4.7 days), the irradiation thus having failed to prolong survival times. None of the transplants showed the usual endothelial cell swelling and differentiation into plasma cells, however, and there was little or no plasma cell proliferation. This is strong evidence for the renal origin of the plasma cells. Irradiation of the recipient likewise failed to prolong homograft survival.

Murray and Holden (1954) (152) reported having irradiated a "small group" of recipients. No details were given, but the authors stated that they were "not impressed with any beneficial results."

Baker and Gordon (1955) (192) treated 9 dogs with 225 r. total body irradiation. Transplantations were carried out 1 to 7 days later. Five of the donors were not irradiated while 4 were. The kidneys were perfused with heparinized saline and the transplant vessels were sutured to the renal vessels of the host. The ureter was united to that of the host over a polyethylene catheter. The white blood cells count in the 4 dogs in which it was measured was 500 or less at the time of transplantation and bleeding was profuse. One dog died 3 days after transplantation and the transplants in the other 8 animals functioned for 2 to 8 days (average 4 ± 1.5 days). Function in the control series showed a range of 1 to 5 days (average 3 ± 1.0 days). The tubular and, to a lesser extent, the glomerular structures were completely destroyed. In contrast to Dempster's transplants, lymphocytic infiltration was reported. More experiments of this type would seem to be indicated, particularly ones in which bone marrow transplantation is accomplished prior to renal transplantation.

### Use of Cortisone, ACTH and Nitrogen Mustard

Persky and Jacobs in 1951 (193) used cortisone and ACTH in an attempt to prolong the survival of renal homografts in dogs. They failed to note any improvement in function or survival as a consequence of this treatment.

Baker and his associates (1952) (173) administered cortisone and nitrogen mustard to 6 dogs and combined this treatment with a splenectomy in 4 others. The duration of renal function in 5 controls was 1 to 5 days with a mean of 3.0 ± 1.0. The transplants in the animals receiving cortisone plus nitrogen mustard functioned for 4 to 20 days with a mean of 13.5 ± 5.2 while those in animals in which splenectomy was added functioned for 5 to 20 days with a mean of 10.2 ± 3.3. The donor kidneys were perfused with a saline-heparin solution, and the transplant vessels were anastomosed to the renal vessels of the host, the ureter being implanted into the bladder. The nitrogen mustard was given in doses of 0.5 mg. per kg. until a leukopenia was achieved in which the polymorphonuclear leukocyte count was reduced to 20 per cent or less of the starting value. All the transplants showed the usual microscopic picture including "lymphocytic infiltration." There was a significant prolongation of function when the host received nitrogen mustard plus cortisone, with or without splenectomy. Nitrogen mustard alone failed to prolong transplant function which in this instance ranged from 1 to 8 days with a mean of 4.5 ± 2.7. The authors pointed out that these results may be due to the poor nutritional status of the treated dogs.

Dempster in 1953 (181) studied the effect of cortisone on renal homotransplants in dogs. The cortisone was administered orally in divided doses totalling 150 to 200 mg. per day beginning one week prior to transplantation. The effect was observed both on primary and secondary transplants and the donor received cortisone for the entire time until the secondary transplantation was done. Dempster found that the plasma cell proliferation was reduced or abolished when the kidney donor was treated with cortisone prior to transplantation. The vascular endothelial reaction was completely abolished. There was a slight reduction of vascular spasm, which failed to prolong the life of the kidney but allowed some corti-

cal flow and good renal flow. Some factor closely related to spasm initiated anuria, which heralded the disintegration of the kidney. Spasm of the glomerular capillaries was prevented in all secondary transplants, but this did not affect survival or reduce the widespread parenchymal damage. These results indicate that the plasma cell proliferation, endothelial changes, and spasm of the glomerular capillaries were all manifestations of the reaction of the transplant against the host, and that they could be reduced or abolished by cortisone pretreatment.

deKlerk and his coworkers (1954) (175) studied the effect of cortisone on canine renal homografts. The right kidney was removed and 5 to 10 days later the animals were anesthetized in pairs and the left kidney of each was transferred to the neck of the other. The only kidney, therefore, was the transplant. The procedure was carried out in 25 dogs, with 3 operative deaths. In 3 cases, one normal kidney was left *in situ*. Of the 22 animals surviving the operation, 16 were controls and 6 received cortisone pre- and postoperatively in doses of 10 mg. per kg. per day. The longest survival time in the control group was 21 days. In only 3 cases was there no evidence of thrombosis of the pedicle. At postmortem examination the glomeruli appeared uninvolved in any pathologic process, while the tubules showed cytoplasmic vacuolation, desquamation, and loss of definition of all elements. In only one case was there no round cell infiltration—in the cortisone-treated dog which survived for 21 days. The other cortisone-treated animals survived for 3 to 5 days and all but one had arterial thrombosis. It is difficult to evaluate the histologic findings in these experiments, because the end-point used was the death of the animal. It seems that cortisone did not improve the function or survival of the transplant, and that in only one case was round cell proliferation prevented.

Murray and Holden (1954) (152) reported that they administered cortisone (the dose was not stated) to 9 dogs receiving homografts. In all cases there was complete necrosis of the transplanted kidney with plugging of both arteries and veins. No details were given.

Darmady, Dempster and Stranack in 1955 (152) did microdissections of the tubules in transplanted kidneys, as mentioned earlier. These studies were also carried out in two homotransplants from cortisone-treated dogs. The cortisone had apparently 1) abolished or reduced the intimal and vascular reactions, 2) improved cortical blood flow after onset of anuria, and 3) abolished the narrowing of the glomerular tubular neck, which was seen in the untreated homotransplants.

Archibald and Cawley (1956) (176) performed homotransplantations in 5 dogs, 2 of whom were also treated with ACTH. The other 3 animals served as controls. Treatment with ACTH was begun immediately after transplantation in one animal and on the sixth day in the other animal. The 3 control transplants survived for an average of 4.5 days, while the 2 ACTH-treated homografts functioned for 10 days. The latter did not differ microscopically from the controls. The authors concluded that ACTH doubled the survival time of the homograft, a conclusion which hardly seems justified in view of the few data and the fact that the functional period of the homografts in the ACTH group was no longer than that occasionally observed by many workers in untreated homografts.

The results obtained in attempts made to prolong renal homograft survival and function with the use of cortisone and ACTH may be summarized as follows: 1) No significant prolongation was noted when either the recipient or donor was treated with cortisone or ACTH. 2) When cortisone was given to the recipient in combination with nitrogen mustard, a significant prolongation of function was noted. 3) The plasma cell proliferation, endothelial changes, and vascular spasm could be reduced or abolished by pretreatment of the donor with cortisone without, however, increasing the functional period of the transplant.

### Lymphatic Isolation

Hume and Egdahl (1955) (167) performed a series of experiments in which they isolated the homotransplanted kidney in a watertight plastic sac, thus removing it from contact with the surrounding tissues and regional lymphatics. The rationale for these experiments was based on the observations that (a) homografts can persist for long periods of time in areas devoid of lymphatic drainage (193–195), (b) regional lymph nodes contain higher agglutinin titers than other nodes after the peripheral injection of antigen (196), (c) an intradermal, and thus intralymphatic injection of homologous leukocytes has a much greater immunizing effect than an intravenous injection (197), (d) following the transplantation

of homologous tumor cells a heightened resistance to the growth of these cells can be passively transferred to other animals of the same strain by grafts of regional lymph nodes draining the site of tumor transplantation, but not by grafts of distant lymph nodes (198) and (c) immunity to homologous grafted skin can be passively transferred to another animal of the same strain by grafts of regional lymph nodes and to a lesser extent, of the spleen (199) Isolation of the transplant from the regional lymphatics was carried out to determine whether this would decrease antibody formation against it and thus prolong survival.

Such was not the case. The isolated kidneys, which were in contact with the host only through the blood stream were rejected in the same manner and after the same interval as non-isolated renal homotransplants. It would thus appear that renal homotransplant antigens can be conveyed to the site of host antibody production by the intravenous route in sufficient quantity to incite an effective antibody response.

**Cross-circulation Experiments**

If a renal homotransplant can incite the production of circulatory anti-transplant antibodies by the host, then it might be possible by means of cross-circulation, to demonstrate the presence of these antibodies. Experiments utilizing cross-circulation were carried out by Egdahl and Hume in 1956 and 1957 (185 186) All transplants were made in the renal fossa with anastomosis of the transplant vessels to the renal vessels of the host and the performance of a skin ureterostomy so that all urine elaborated by the transplant could be collected and analyzed. The end-point of transplant function was defined as the time at which the 24-hour urine volume fell to 50 cc. or less or gross blood appeared in the urine whichever occurred first. The studies may be summarized as follows

1) In 3 cases the homotransplant recipient (dog) was exsanguinated into a sterile bottle on the day the transplant ceased functioning The blood was centrifuged and the plasma was withdrawn. The plasma, totaling 500 cc., was then infused into the renal artery of the remaining kidney of the donor after which the circulation to this kidney was restored. A biopsy of the kidney was taken just before the infusion and repeat biopsies were made at intervals thereafter No changes occurred in the remaining donor kidney as a consequence of this infusion.

2) In two recipients, the spleen was removed at the time of cessation of homograft function. An extract was made from the spleen and this was injected into the renal artery of the remaining donor kidney as above. Again, no changes occurred in the remaining donor kidney

3) The host and donor aminals were put into cross-circulation either during the period of rejection of the transplant or immediately after its rejection and removal. In one experiment the animals were cross-circulated on 5 of the 6 days the transplant functioned In another 3 experiments cross-circulation was maintained for 6 to 24 hours at the height of the homotransplant rejection. In another experiment the cross-circulation was carried out for 20 minutes directly into the donor's renal artery A total of 9 cross-circulation experiments was carried out between host and donor at some time after transplantation. The rate of blood flow was about 500 cc per min. None of the remaining donor kidneys showed any significant change.

4) In 4 experiments a single transfusion of 300 cc. of blood from the prospective donor was given to the prospective host from 7 to 14 days before transplantation. The subsequent transplants behaved like typical primary transplants.

5) In 15 experiments the prospective donor and recipient were cross-circulated prior to transplantation. The results were discussed earlier under *Secondary Renal Homotransplants* To summarize 11 of 15 transplants showed secondary reactions, and two additional ones had thromboses of both artery and vein, which was not seen in primary transplants. Two of the 15 had primary reactions—these were performed 9 and 21 days after cross-circulation. The secondary reactions occurred in transplants between animals previously cross-circulated for periods as short as 5 minutes and as long after the cross-circulation procedure as 28 days.

6) In 4 experiments cross-circulation was started immediately after transplantation. In one of these the pair of dogs had been immunized by pre-cross-circulation for 68 hours 10 days prior to transplantation. The cross-circulation was maintained for 3 days after transplantation. Biopsy at this time showed a kidney of normal appearance The cross-circulation was then stopped. Two days later the transplant ceased functioning and at removal both artery and vein were thrombosed. The other 3 experiments were done in dogs not previously immunized and the cross-circula

tion was carried out for 2, 3, and 4 days respectively. Only minimal changes were noted in these transplants.

Certain conclusions may be drawn from these experiments. 1) When plasma or spleen extract from the host which had rejected the transplant was injected directly into the renal artery of the remaining donor kidney, it failed to damage it. Furthermore cross-circulation of the host's blood into the renal artery of the remaining donor kidney for a period of 20 minutes likewise failed to damage the kidney. This is what might be expected, because Simonsen found, as mentioned earlier, that a secondary transplant was not injured after exposure to the host for one hour before retransplantation (151), although when a transplant was exposed to a host immunized against A+ antigens, it *was* destroyed after a stay of one hour (189). The second kidney of the donor was acting as a secondary transplant exposed to the host for brief periods up to 20 minutes, after which exposure was withdrawn and the kidney remained in the donor (retransplant). It is not surprising, therefore, that no changes occurred in it. 2) More surprising were the results with the prolonged periods of cross-circulation of the entire animal. A very brief cross-circulation served to immunize, so that tissue antigens must have been present in the blood stream. Prolonged cross-circulation of the *immunized* host and the donor (6 to 24 hours) failed to injure the donor kidney. Several explanations of this are possible, but the two most likely are that (*a*) antigens in the blood of the donor animal neutralized the antibodies of the host before they could fix in effective profusion on the kidney or other organs, or (*b*) some protective substance is present in the blood of the donor animal which, while circulating through the tissues, prevents the fixation of host antibodies on the tissues. 3) Most surprising of all was the finding in one experiment that an immune host failed to alter a transplant while the host and donor were joined in cross-circulation. The two explanations just given above could again account for this phenomenon. Further experiments of this type might yield additional information.

Dempster (1) removed blood from three kidney transplant recipients and injected 150 cc. of plasma into the donor animals. No abnormality of the remaining donor kidney was noted.

Two other reports are worth mentioning in this section, although the techniques used were not strictly comparable to those under discussion. Kamrin (200) grafted renal tissue from one rat into the kidney of a second. The grafts sloughed in 7 to 11 days. If the rats were put into parabiosis at the time of grafting, the grafts survived (provided the parabiosis was successful). Subsequent separation led to rejection of the graft.

In 1955 Lang, Dammin, and Miller (201) performed parabiosis and pseudoparabiosis (the union was prevented) in rats. A kidney of one rat was then transexplanted into the subcutaneous tissue of its parabiotic partner. In pseudoparabiosis the transexplanted kidney appeared indistinguishable from one explanted into the animal's own subcutaneous tissue. In true parabiosis, on the other hand, in which there was blood circulation between the two animals, the transexplanted kidney showed cellular infiltration obliterating the capsule and extending into the subcapsular cortex. Pan-arteritis was present in the smaller arteries of the explanted kidney. These changes did not occur if the kidney was left in its normal site in parabiosis. The changes were thought to be due to contact plus exchange of blood and body fluids. These very interesting results would seem at first to be contradictory to those found in one experiment by Egdahl and Hume. However, one might explain this difference by hypothesizing that the subcutaneous contact at the edge of the parabiotic flaps in the rats had immunized the "host" against the "donor" so that the subcutaneous contact between the transexplant and the "host" permitted the direct invasion of the explant by host antibodies (since this did not occur if the kidney remained *in situ*). The "cross-circulation" between the two may have been too slow to provide effective antibody neutralization. The pseudoparabiotic animal, not being immunized, failed to show this response. If one accepts this hypothesis, however, it becomes very difficult to explain Kamrin's results. Clearly more work should be done in this regard.

## Effect of Pregnancy

The pregnant animal tolerates the fetal "homograft" without difficulty. This raises the question as to whether the pregnant animal might tolerate other homografts better than the non-pregnant animal. Whether this idea occurred to the early investigators of renal transplantation is difficult to say, but in any event, two of them performed some renal transplantations in animals which

TABLE 24

*Transplantation in Pregnancy*

| Pregnant Recipient or Host | | | Non-pregnant Recipient and Host | | |
|---|---|---|---|---|---|
| Donor | Host | Survival Time | Donor | Host | Survival Time |
| **Cats by Carrel** | | | | | |
| | | *days* | | | *days* |
| ♂ | Pregnant ♀ | 8 | ♂ | ♂ | 14 |
| Pregnant ♀ | Non pregnant ♀† | 31 | ♂ | ♂ | 10 |
| Pregnant ♀ | Pregnant ♀ | 13 | ♀ | ♀ | 36 |
| Pregnant ♀ | ♂ | 13 | | | |
| **Dogs by Unger** | | | | | |
| ♂ | Pregnant ♀ | 11 | ♂ | ♂ | 17 |
| | | | ♂ | ♂ | 5 |

Abscess

† Parturient 2 days before

happened to be pregnant. The results of these experiments have been assembled in table 24 along with the results obtained by the same authors in concurrent work with non-pregnant animals. To summarize, Carrel in 1908 (23) performed renal transplantations *en masse* in cats. One recipient which was two days postparturient received the kidneys of a pregnant cat and survived for 31 days. Another pregnant recipient received the kidneys of a pregnant donor and survived for 13 days but had an abscess. In another case a male recipient received kidneys from a pregnant donor and survived for 13 days but also had an abscess. Two sets of transplants from male donors to male hosts survived for 10 and 14 days respectively and a female host to a graft from a female donor survived for 36 days. Although this experiment failed to demonstrate prolonged homograft survival in pregnancy it is interesting that both long term survivals were in female to female transfers.

Unger in 1909 (42) performed a renal homotransplantation from a male dog to a pregnant female. The transplant survived for 11 days as against 5 and 17 days for two pairs from male to male animals. Murray and his coworkers in 1955 (202) mentioned transplanting a dog kidney into a pregnant recipient and noted that it was still functioning when the animal was sacrificed at 12 days.

A more systematic investigation of transplantation in pregnancy may be worth pursuing.

## Use of Litter Mates or Puppies

A few experiments have been performed in which litter mates or puppies were used. Mantelli (1913) (54) performed 9 renal homotransplantations in which the kidneys of a newborn puppy were anastomosed into an adult animal, utilizing the aorta and vena cava of the transplant. Mantelli was attempting to determine whether kidneys of stillborn animals might be useful. All 9 grafts failed.

Dederer in 1920 (60) performed a homotransplantation from one puppy to another of the same litter. The recipient died of distemper on the twenty-sixth day. A P.S.P. test done on the twenty-sixth day was said to show excretion of the dye from the transplanted kidney.

Murray and his coworkers in 1955 (202) homotransplanted in a 22-day-old puppy a kidney which had been ischemic for two hours. It was still functioning at sacrifice 12 days later but failed to sustain life.

It may be that renal transplants survive longer than usual when exchanges are among litter mates but more data on the matter are necessary.

## Properdin

Properdin is a $\beta$-euglobulin with a molecular weight 20 times that of albumin, which depends on complement and magnesium + for action. Because this substance is part of the natural defense mechanism of the blood, Hudav and Persky in 1957 (203) postulated that it suppres-

sion might allow renal homograft survival. Blood levels of properdin were depressed with zymosan, but the life of the renal homografts was not prolonged (survival times ranged from 1 to 8 days). The animals became very prone to infection, and it was difficult to avoid sepsis.

It was reported by Good and his associates in 1957 (204) that preliminary studies had shown at least normal concentrations of properdin to be present in the sera of agammaglobulinemic patients. Since these patients are able to accept homografts, it does not seem that properdin alone is responsible for homograft rejection.

### Other Attempts to Alter Transplant Rejection

#### *Ischemia and Uremia*

Hume and his associates have found that renal transplants in man functioned longer than those in dogs and other experimental animals (205). These transplants were always placed in uremic hosts and after a variable period of ischemia—usually longer than that required in dog homotransplantations. Lang, Murray, and Miller (206) tried increasing the length of the ischemic interval and making the host uremic to see whether this would alter homograft survival. In 4 dogs the survival time was not increased by these measures.

#### *Refrigeration*

Several authors have tried cooling or refrigeration of renal homografts. Avramovici in 1924 (63) transplanted dog kidneys which had been preserved in cold temperatures for varying periods of time up to 30 hours. One kidney which had been thus preserved for 8 hours was then homotransplanted, one of the host's kidneys being removed. Fourteen days later the other kidney of the host was removed. The animal was said to have lived for 36 more days. The lack of detailed studies and the failure of other investigators to approach the level of success with renal homotransplants reported by Avramovici make these results highly suspect.

Oulot in 1948 (123) put dog kidneys on ice at 4°C.—*for as long as* 8 days—before homotransplanting them. He obtained uniformly poor results, all kidneys infarcting as soon as the blood flow was restored.

Lefèbvre in 1951 (127), using dogs, removed the kidney from the donor and perfused it with saline solution at a pressure of 140 to 160 mm. of mercury until the renal vein effluent was clear. The kidney was placed in physiologic fluid on ice for 3 to 24 hours, sometimes being cooled to as low as 1°C. It was then transplanted to the neck. The transplant diuresed less rapidly than an immediate transplant, and sometimes a bilateral nephrectomy was done, or urea was injected to stimulate diuresis. The best urea concentration was five times that of blood. The transplant secreted bilirubin and urochrome, and had a normal chloride output and oxygen consumption. Lefèbvre concluded that the kidney could be kept at a low temperature up to 24 hours and still show a return of function, although recovery was always less than normal. These experiments were all short term, over a few hours, so that no information is available as to whether recovery would have been progressive.

Murray and Holden in 1954 (152) cooled some autotransplants and found that the period of ischemia could be lengthened by cooling the kidney to temperatures ranging from 4° to 10°C. Archibald and Cawley in 1956 (170) cooled dog auto- and homotransplants to 4°C., refrigerating some for as long as 24 hours. A total of 4 auto- and 8 homotransplants were treated thus. The autotransplants secreted a poor quality blood tinged urine for an average of 7.0 days, the longest survival time being 14 days. They were almost completely destroyed histologically. The homografts secreted only small amounts of bloody urine for an average of 3.5 days. Non-cooled autotransplants functioned for as long as 5 months as the only kidney. Mitchell and Woodruff in 1957 (171) demonstrated that the ischemic period of renal autografts in sheep could be lengthened by local cooling of the kidney.

It seems from these results that cooling of the renal transplant permits a somewhat longer period of ischemia, but that storage for more than three hours at low temperatures leads to severe renal damage in the experimental animal.

### Type of Animal Used

The duration of renal homotransplant function and survival depends to some extent on the species of the animal. Reports indicate that transplants are most effective in cats and least effective in dogs. Goats occupy an intermediate position. It is interesting to note that renal homotransplants in goats do not show the endothelial swelling so characteristic of canine homotransplants.

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Several authors have tried cooling or refrigeration of renal homografts. Avramovici in 1924 (63) transplanted dog kidneys which had been preserved in cold temperatures for varying periods of time up to 30 hours. One kidney which had been thus preserved for 8 hours was then homotransplanted, one of the host's kidneys being removed. Fourteen days later the other kidney of the host was removed. The animal was said to have lived for 36 more days. The lack of detailed studies and the failure of other investigators to approach the level of success with renal homotransplants reported by Avramovici make these results highly suspect.

Oudot in 1948 (123) put dog kidneys on ice at 4°C—for as long as 8 days—before homotransplanting them. He obtained uniformly poor results, all kidneys infarcting as soon as the blood flow was restored.

Lefèbvre in 1951 (127) using dogs removed the kidney from the donor and perfused it with saline solution at a pressure of 140 to 160 mm. of mercury until the renal vein effluent was clear. The kidney was placed in physiologic fluid on ice for 3 to 24 hours, sometimes being cooled to as low as 1°C. It was then transplanted to the neck. The transplant diuresed less rapidly than an immediate transplant, and sometimes a bilateral nephrectomy was done, or urea was injected to stimulate diuresis. The best urea concentration was five times that of blood. The transplant secreted bilirubin and urochrome and had a normal chloride output and oxygen consumption. Lefèbvre concluded that the kidney could be kept at a low temperature up to 24 hours and still show a return of function although recovery was always less than normal. These experiments were all short term, over a few hours, so that no information is available as to whether recovery would have been progressive.

Murray and Holden in 1954 (152) cooled some autotransplants and found that the period of ischemia could be lengthened by cooling the kidney to temperatures ranging from 4° to 10°C. Archibald and Cawley in 1956 (176) cooled dog auto- and homotransplants to 4°C, refrigerating some for as long as 24 hours. A total of 4 auto- and 8 homotransplants were treated thus. The autotransplants secreted a poor quality blood tinged urine for an average of 7.6 days, the longest survival time being 14 days. They were almost completely destroyed histologically. The homografts secreted only small amounts of bloody urine for an average of 3.5 days. Non-cooled autotransplants functioned for as long as 5 months as the only kidney. Mitchell and Woodruff in 1957 (171) demonstrated that the ischemic period of renal autografts in sheep could be lengthened by local cooling of the kidney.

It seems from these results that cooling of the renal transplant permits a somewhat longer period of ischemia, but that storage for more than three hours at low temperatures leads to severe renal damage in the experimental animal.

### Type of Animal Used

The duration of renal homotransplant function and survival depends to some extent on the species of the animal. Reports indicate that transplants are most effective in cats and least effective in dogs. Goats occupy an intermediate position. It is interesting to note that renal homotransplants in goats do not show the endothelial swelling so characteristic of canine homotransplants

This particular change has not been definitely reported on in cats. As will be discussed subsequently, man seems to be a better subject for renal transplantation than any experimental animal.

## HYPERTENSION AND RENAL TRANSPLANTS

Renal damage may give rise to hypertension, and the problem is therefore pertinent to the subject of renal transplantation. Three questions at once suggest themselves: 1) Will a successful transplant reduce an existing hypertension even though the diseased kidneys remain in place? 2) Will a homotransplant *create* hypertension in the normotensive host? 3) Will a homotransplant reduce an existing hypertension when both host kidneys are absent? At least partial answers to the latter two questions are available in experimental data, and all three questions are more fully answered in the clinical data presented later.

As mentioned earlier, Glen, Child and Heuer in 1937 and 1938 (111, 112) in autotransplanting kidneys to the inguinal area in dogs demonstrated that constriction of the artery of the transplants led to hypertension. Houssay and Fasciolo in 1938 (113) showed that the graft of a partially ischemic kidney in a normal dog whose kidneys were excluded produced hypertension, while the graft of a normal kidney did not. Braun-Menendez and Fasciolo in 1940 (114) showed that constriction of the artery of a normal kidney grafted to the neck produced hypertension in 2 to 7 minutes. Brull and Dumont (1942) showed that a normal transplant would reduce the blood pressure of a nephrectomized dog (92), while an ischemic transplant would not (93). Fasciolo and Taquini in 1950 (116) found that incomplete ischemia of a kidney transplanted to the neck produced an increase in renin content.

Scott and Bahnson (1951) (148) produced hypertension by creating experimental coarctation in dogs. After the establishment of hypertension one kidney was transplanted to the neck and the other kidney was removed in 8 dogs. In another 5 dogs one kidney was transplanted to the iliac vessel and the other kidney was removed. Five of the 8 transplants to the neck were successful, surviving for 2 weeks and 1, 2, 3 and 4 months respectively. In all 5 cases there was a prompt reduction in the pressure which had been elevated as a consequence of the coarctation. One of the 5 animals into which the kidney was transplanted to the iliac vessels survived. There was no change in its hypertension over the period of 4½ months during which it was observed.

Muirhead in 1952 (207) and he and his associates in 1956 (155) produced hypertension in dogs by bilateral nephrectomy. The dogs were maintained by peritoneal lavage for 12 to 14 days during which time hypertension and moderate azotemia developed. A kidney was then homotransplanted to the neck vessels. The azotemia returned to normal and the blood pressure dropped precipitously. These changes continued for several days and were followed by the reappearance of hypertension and azotemia as the kidney failed. The ability of the homograft to reduce blood pressure was distinct from its ability to excrete nitrogenous wastes or concentrate the urine. Shpuga in 1956 (150) also found that a transplant to the neck reduced the blood pressure in experimental hypertension. Simonsen and his coworkers (151) noted the development of hypertension in some of the recipients of homologous renal transplants in dogs. These transplants were probably partially ischemic, however, because of the use of plastic prostheses in making the arterial anastomoses.

These studies indicate that 1) a successful homotransplant will reduce hypertension if the diseased kidneys are removed or excluded from the circulation, and will perhaps do so to some extent even if the kidneys are not removed, and 2) a homotransplant will not create hypertension in the normotensive host unless it is partially ischemic.

The relationship of homografts and isograft to hypertension will again be alluded to in the section on clinical studies.

## TWINNING AND COMMON PLACENTAL CIRCULATION

In 1916 Lillie (208) showed that most cattle twins are dizygotic but with a large single chorion, resulting from a fusion of the blood vessels from the two developing chorions. Either fetus can be injected from the other. A constant interchange of blood occurs between the two. When the two embryos are of a different sex a suppression of the reproductive system of the female occurs as a consequence of the effect of male hormone from the twin. This results in sterility of the female

"freemartin A fertile and normal freemartin results if the circulation of the two chorions fails to fuse.

This very important observation led Owen in 1945 (209) to study the inherited cellular antigens in more than 80 pairs of bovine twins He found that the majority of the pairs had the same blood type, despite the relative rarity of identical (monozygotic) twins in cattle. Identity of blood types between non twin siblings was infrequent. This suggested that the common placental circulation between the genetically dissimilar (dizygotic) twins was responsible for the sharing of antigens which led to the phenotypic identity of blood types. Other immunologic evidence was given to suggest that embryonal cells ancestral to the erythrocytes of the adult animal became interchanged between the twins. These cells then became established in the hematopoietic tissues of the co-twin and provided a source of blood cells distinct from those of the host. A chimera was thus produced.

Billingham, Brent and Medawar in 1955 (210) showed that dizygotic cattle twins would accept skin homografts from each other while they would not accept them from full siblings of separate birth. This confirmed the interchange of tissue antigens through the common placental circulation. Simonsen in 1955 (189) reported an experiment he did in collaboration with Gammeltoft and Sorensen in which a kidney was removed from one dizygotic cattle twin and transplanted to the other's neck. One of the host's kidneys was removed. The transplant was still secreting an abundant volume of urine 16 months later and the creatinine clearance was 50 per cent of that of the normal kidney

These studies indicate that renal homotransplants are possible between animals which have been exposed to each other's antigens *via* the blood stream alone, during embryonic life. As the work of Egdahl and Hume indicated cross-circulation, even in adult life is an excellent way to produce immunity or perhaps to neutralize circulating anti transplant antibodies with circulating donor antigens.

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## II STUDIES IN MAN

### HISTORICAL REVIEW

A few human renal homotransplants and heterotransplants were studied in which no significant function was conclusively demonstrated Princeteau in 1905 (1) presented a case of a child with osteomyelitis of the upper end of the femur albuminuria, anasarca, vomiting and a urinary output of only 20 cc. per day The child was operated upon, and a left nephrotomy was done. Two pieces of the kidney of a rabbit were inserted into the wound The immediate results were excellent. The urinary volume increased and vomiting stopped Fifteen days later the urinary output was one liter a day On the sixteenth day the child died of pulmonary congestion.

It is apparent from this account that unwarranted optimism and failure of objective and critical observation were features not only of the early experimental studies but characterized clinical reports as well

Jaboulay in 1906 (2) heterotransplanted the kidney of a pig to the vessels of the anticubital fossa of a young woman dying of uremia. No function was noted. The transplant of a goat kidney under similar circumstances met with no greater success.

Unger in 1910 (3) grafted an ape kidney into a 21 year-old girl with renal disease. The aorta and vena cava attached to the kidney were anastomosed to the femoral artery and vein. The graft did not function.

In 1913 Schönstadt (4) transplanted a monkey kidney to a young woman anuric from sublimate poisoning. The kidney was placed on the arm and death followed a few hours later.

After these initial ill-fated human heterotransplants a few human renal homotransplants were employed in which no significant function was demonstrated. In the first two instances the transplants were intended as a temporary aid to tide the patient over an episode of acute anuria. Voronoy in 1930 (5) transplanted a kidney into the groin of a patient with bichloride of mercury poisoning but the patient died 48 hours later and no conclusions were possible. Voronoy recognized the possible value of reticuloendothelial blockade by the mercury and hoped by this means to escape the inevitable homograft rejection which he believed was due to antibodies formed in the reticuloendothelial system. Landsteiner and Hufnagel in 1945 (6) transplanted a kidney from a cadaver to the brachial artery and cephalic vein of a young woman in acute renal failure. The patient's own kidneys resumed function a few hours after the transplantation, and the transplant was removed after 48 hours without having secreted any significant amount of urine.

Lawler and his associates in 1950 (7) removed a kidney from a woman with polycystic kidney disease and anastomosed the kidney from a cadaver onto the patient's own renal artery and vein. The ureter was anastomosed to that of the recipient. No separate collections were made from the two ureters, and the only renal function study done was the injection of indigo carmine dye parenterally on the fifty-second day. It was stated that the dye emerged from both the ureters but in poorer concentration from the side of the transplanted kidney. The transplant was removed 9½ months after implantation and the kidney at this time was found to be 4 by 3 by 2 cm. in size with a complete absence of the ureteral and pelvic structures. It was not producing urine (8). Lawler's case was summarized by Day (9).

Servelle and his coworkers (10) reported on one human homotransplant and Dubost and his associates (11) reported on two transplants at a meeting of the Medical Societies of Paris Hospitals in January 1951. All transplants were made in the pelvis of the recipient and the vessel were anastomosed to the iliac vessel. The kidney transplanted by Servelle and his coworkers 14 days before the meeting was subsequently reported (12, 13) to have reached an output of 600 cc. by the nineteenth day, at which time the patient suddenly died. The transplant was said to show infarction with lymphocytic infiltration and acute nephritis.

The two transplants reported by Dubost and his associates had been performed 14 and 3 days before the meeting. The first kidney was subsequently reported (14) to have secreted a maximum of 30 cc. of urine in 24 hours and the patient died on the sixteenth day. The second transplant secreted only a few drops. There was no indication that these kidneys were actually performing any significant amount of work.

Küss, Teinturier and Milliez were reported by Lawler and his associates (8) to have performed 10 homotransplantations in man, several of which "functioned for several months," but in their publication (15) they described only five and in a personal communication to the author (16) Küss reports having done only one more. Regarding the five published cases, one patient died during surgery, and in another case the graft was removed 48 hours after the transplantation. A third patient developed necrosis of the ureter and the transplant excreted small amounts of urine for only 18 days. The patient died on the twentieth day. A fourth transplant developed necrosis of the ureter on the twentieth day. The maximum output was 72 cc. per 24 hours, on the tenth day. In the final subject the maximum 24-hour output was 45 cc. The ureter became necrotic on the twentieth day and the patient died on the thirty-fifth day. An extensive infarction of the kidney was found at necropsy. A sixth patient was said to have shown no better function (16). In these cases the transplant was placed in the pelvis of the recipient and an anastomosis was carried out between the hypogastric artery and the renal artery and between the external iliac vein and the renal vein. The ureter was brought out to the skin.

A consideration of a few reported cases of functioning renal homotransplants in man is now pertinent.

## FUNCTIONAL, GROSS AND MICROSCOPIC FEATURES

In 1952 Hume, Merrill and Miller (17) reported that significant function had been achieved in three of six human renal homotransplants for periods of from one to three months. The pathologic changes in these and two additional patients were mentioned in an abstract by Hawn and his associates in 1953 (18) A further brief mention was made of these cases by Hume in 1953 (19)

In 1953 Michon and his coworkers (20) reported the case of a 16-year-old boy who had had a right nephrectomy for traumatic hematoma of the kidney following which absence of the left kidney was discovered On the eighth day of anuria a normal kidney was removed from the patient's mother and transplanted into the pelvis of the patient by an anastomosis with the iliac vessels. The period of anoxia was 55 minutes. A uretero-ureteral anastomosis allowed the patient to void in normal fashion. The mother's blood type was identical to that of the patient. Urine was elaborated from the homograft within two hours after completion of surgery A good urinary volume, reaching 1 5 liters in 24 hours, and good function were maintained for three weeks thereafter with marked improvement in the patient's clinical condition and azotemia. The urea clearance reached 12.5 cc. per minute. The function of the homograft ceased quite suddenly however on the twenty-third postoperative day There had been no preceding hematuria, infection, or mechanical obstruction, but proteinuria had been noted the last few days. The site of the transplant was explored the day after urine flow ceased The transplant was found to be odematous, enlarged, and ecchymotic. The anastomoses appeared to be all right. A biopsy showed numerous bloodless vessels in the glomeruli with marked infiltration of the parenchyma by leukocytes predominantly lymphocytes and plasma cells. There was widespread degeneration of the tubular epithelium, apparently secondary to ischemia of the glomerular capillaries.

The first report of this case was followed by a second by Oeconomos and associates (1953) (21) who discussed some of the technical aspects in more detail This article was summarized in English (22) Hamburger Richet and Antoine in 1954 (23) again reported the case showing photomicrographs of the renal biopsy There were endothelial proliferation and thrombosis present in some of the arteries.

Ducrot and Antoine in 1954 (24) also reported the case, stressing some of the immunologic aspects and it is referred to in some detail in three other articles by Antoine and Ducrot (1954–1956) (25–27) and in one more by Oeconomos in 1956 (28) In 1955 Darmady Dempster and Stranack (29) reported the results of a microdissection of a portion of this kidney supplied to them by Hamburger and Richet. Endothelial changes were similar to those found in dog homotransplants, with differentiation of these cells into plasma cells. The entire proximal tubule was disorganized and atrophic but the lesions of the glomerular tubular neck seen in primary dog homotransplants were not present. Presumably these studies were carried out on pieces of the transplant obtained at autopsy which was eleven days after the transplant ceased functioning

In 1955 Hume and his coworkers (30) reported in detail their experiences with nine cases of renal homotransplantation in the human. Some of these cases had been briefly referred to before Four of the transplants showed significant measurable function, secreting urine for 37 to 176 days. The kidneys were placed in the thigh the renal vessels being anastomosed to the profunda femoris artery and the common femoral vein. The ureter was brought through a stab wound in the skin. Some technical difficulties were encountered in the first few cases because of edema of the skin overlying the kidney In the later cases, however a double-ended skin flap was brought over the kidney and the site from which this was taken was grafted with a split thickness graft so that a loose pocket was made to hold the kidney All of the patients were in the terminal phases of chronic uremia. Since the ureter was brought out to the skin it was possible to collect the urine from this kidney separately and since the transplant was added as a third kidney its function could be compared to both native kidneys which were left undisturbed. Three of the transplants showing prolonged function were from cadavers the fourth was obtained by operative removal from a child undergoing a subarachnoid-ureteral anastomosis The periods of anoxia were 55, 135 180 and 200 minutes. Some of the donors had been in shock for a prolonged time before death as well, thus increasing the period of ischemia. It is not surprising therefore,

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FIG 206 Lateral view of the ureterostomy of one of the human kidneys tran planted by Hume and hi associates pictured on the one hundred and fifth day after transplantation A peristaltic wave may be seen spurting urine out of the end of the ureter

that after transplantation the grafted kidneys went through a period of anuria corresponding to that seen in patients with acute ischemic tubular necrosis. After a period of anuria or oliguria varying between 8½ to 10 days, the transplants began to diurese and to function. The first achieved maximal function between the eighteenth and the twenty-second day with a good volume of secretion for 33 days and a fair volume for 53. It was still secreting to some extent on the seventieth day The second transplant achieved maximal function from the thirteenth to the twenty-eigth day gradually declining from the twenty-nineth to the sixty-fifth day The output dropped markedly from the sixty-sixth to the one-hundred and first day at which time the kidney was removed The third transplant secreted 1700 cc during the first 24 hours and 153 cc during the second it then became oliguric for 12 days. After diuresis this kidney achieved maximal function from the fourteenth to the twenty-sixth day and then declined until the thirty-seventh day



FIG 207 A retrograde pyelogram of one of the human renal homotran plants done by Hume and his a sociates taken on the eighty third day There is no evidence of obstruction or di tortion of the calyces pelvi or ureter

The fourth transplant began to diurese on the nineteenth day reaching on the thirty-seventh day an effective function, which continued to *improve at least as late as the one-hundred and* fifty-third day when the la t tests were carried out. The latter showed that the urea clearance was 8.7 cc. per minute the inulin clearance was 8 9 cc per minute per 1 73M² the PAH clearance was 36 cc. per minute per 1 73 M² and the average urine volume was 2.3 cc per minute The blood urea nitrogen had come down to 34 mg per cent from a high of 244 mg. per cent the serum $CO_2$ was 20 mM per L. the serum sodium was 135 mEq per L. and the hematocrit was 41 The urine was ejected from the end of the skin ureterostomy by vigorous peristaltic waves (fig. 206) and the patient, who had been living at home was feeling well. On the one-hundred and sixty-second day the patient noted a slight swelling of the kidney transplant area in the right thigh. This had increased by the following day and the transplant urine contained many white blood cells for the first time By the one-hundred and sixty-eighth day the urinary volume began to decrease and the blood urea nitrogen began to rise The patient died on the one-hundred and seventy-sixth day after the transplantation and 14 days after the first sign of failing function of the transplant His blood urea nitrogen had risen to 260 mg per cent at the time of death indicating the inability of his own diseased kidneys to maintain him once the tran plant had ceased to function

The normal architecture of the calyces pelvis and ureter were well maintained for many days after the transplantation Figure 207 is a retrograde pyelogram of a kidney transplanted into the thigh 83 days previou ly Essentially normal configuration of the collecting pathway is present

Several similarities were *noted among* the 4

transplants in the series showing measurable function 1) All of the kidneys were small and had vessels which were equal to or smaller than those of the femoral vessels to which they were anastomosed. 2) All patients received testosterone in the immediate postoperative period and for 27 to 62 days thereafter 3) The blood types of donor and recipient were the same and the bloods cross-matched. 4) All kidneys went through a period of anuria from 8¼ to 19 days in duration before beginning to secrete urine—undoubtedly a consequence of the prolonged ischemia prior to transplantation. 5) During the period of maximal function each of the four transplanted kidneys developed function which was better than that of the recipient's own kidneys. The maximum urine volumes in the four cases were 600+ cc., 1400+ cc. (some was lost in collection), 2800 cc. and over 3000 cc. in 24 hours.

The first three functioning transplants ultimately became heavily infected The main renal vessels remained patent in one patient, but had become thrombosed by the terminal stage in the other two patients. The gross appearance of the transplant at the time of removal varied somewhat, depending on the degree of infection which accompanied or followed its destruction by immunizing processes. One of the kidneys removed when the patient died 37 days after the transplantation still looked fairly normal except for slight enlargement and some mottling of the surface. There was recognizable cortical architecture and the artery and vein were patent. In other instances when the kidney had been left in place after function ceased, there were areas of infarction and cortical abscesses, and the normal renal architecture had been completely obliterated.

The final destructive processes appeared to consist of cortical ischemia, tubular degeneration, and interstitial edema and round cell proliferation. This was followed by scattered thromboses of the small intrinsic renal vessels with associated focal infarcts The glomeruli appeared to be relatively uninvolved except by ischemia until the destruction of the kidney was nearly complete.

The fourth functioning transplant was placed in a polyethylene bag at the time of transplantation, but this was found at necropsy to have become torn. Grossly the transplant was edematous and somewhat increased in size. The cut surface was pale with accentuation of the renal architecture.

Effective function of this kidney was not achieved until the thirty-seventh day—a time at which the function of the other transplants was already declining—and the function persisted and continued to improve for over 5½ months from the time of transplantation. The microscopic findings in this kidney were somewhat different from those in the other three functioning transplants The tubules showed signs of acute ischemic nephrosis, and there were interstitial edema and focal plasma cell proliferation as in the other cases. There was, however no significant infection, no thrombosis no infarct, and no necrosis Many blood vessels had developed a severe degree of endothelial thickening (figs. 208 and 209) With the exception of the portion of the transplant immediately subjacent to the polyethylene bag the glomeruli appeared somewhat ischemic but otherwise essentially normal (figs. 210 and 211)

It is of interest that these human renal homotransplants functioned much longer and appeared



FIG 208 A low power microscopic view of one of the intrinsic vessels in a homotransplanted kidney reported by Hume and his associates This transplant functioned for 176 days Note the marked intimal thickening which has progressed almost to the point of complete occlusion of the vessel The inner portion of intima shows dense sclerosis and the outer is filled with lipid laden macrophages The medium is thinned



FIG 209 A high power magnification of the same transplant shown in figure 208 It shows the wall of one of the vessels of the homotransplanted kidney In the lower left hand corner is the inner sclerotic layer of the thickened intima In the upper right hand corner is the medium Between these two is the marked lipid infiltration of the outer half of the intima

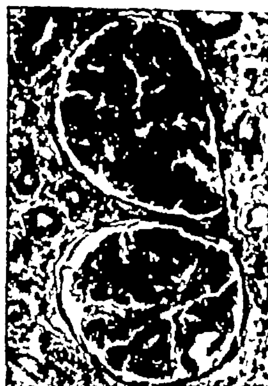


FIG 210 The same case shown in the preceding two figures The glomerular tufts appear ischemic but otherwise fairly normal There are no proliferating changes present in the glomerular capsule The epithelium of the tubules is low and the nuclei are variable in staining There is interstitial edema and polymorphonuclear and round cell infiltration This kidney functioned for 176 days

much better histologically than did transplants performed in experimental animals.

Murray and Holden in 1954 (31) reported four clinical cases of renal homotransplantation. The patients died without significant function of the transplant in three of these cases. In the fourth case the transplanted kidney was thought to be viable and effective fifteen months after transplantation. However since the ureter was put *into the bladder and since the patient's own* kidney also emptied into the bladder no function studies were available and there is no conclusive evidence that the kidney is alive and functioning. No biopsy was done

## ISOTRANSPLANTS IN HUMAN TWINS

It was mentioned in the section on experimental studies that a permanently successful renal homotransplantation had been achieved between dizygotic cattle twins with a common chorion. Previous studies had shown that skin grafts would also survive permanently under these circumstances. Grafts between dizygotic twins who do not have intermingling of the placental circulation are not permanently successful. Brown showed in 1937 (32) that skin isografts between identical twins are permanently successful. Since of course the antigenic mosaic of monozygotic twins is identical, transplants between such pairs will be referred to here as isotransplants in order *to distinguish their results from those of other* homotransplants

In 1956 Murray Merrill and Harrison (33) in one report and these authors in association with Guild in another report (34) described a successful renal transplant between identical twins. The twins were 24-year-old males and *the recipient* suffered from severe glomerulonephritis. The transplanted kidney was ischemic for 82 minutes. It was placed in the pelvis the artery being anastomosed end-to-end to the hypogastric artery and the vein, end-to-side to the iliac vessel. The ureter was inserted into the bladder. The kidney began to secrete urine at once and



FIG 211 A high power view of one of the glomeruli of the same case shown in the preceding figure It shows no essential changes from normal

achieved essentially normal function (Bricker and coworkers (35)) The recipient's own kidneys were removed, and the patient has remained healthy and has had good renal function for 3 years. Other reports of the first case have appeared (36–39) and further discussion of it will be presented here subsequently

Since this manuscript was prepared an article has appeared summarizing the total experience of Murray Merrill, and Harrison (39A) with isotransplants in twins. Transplantation was carried out in 8 pairs of twins. One was a technical failure Of the remaining 7 pairs two transplants developed the disease of the host (commented on later) and the recipients have died In the first 5 cases the host's own kidneys were removed individually at separate operations several weeks after transplantation. In the sixth case the patients died before host kidney removal. The seventh case of isotransplantation in twins was performed by Hume Schilling and their associates (39B) and in this case both of the host's kidneys were removed simultaneously 3 weeks after the transplantation The eighth case (Murray's seventh) also had a simultaneous bilateral nephrectomy and in the ninth case, reported in an addendum by Murray and his associates (39A) the patient had a left nephrectomy 4 days prior to transplantation and a right nephrectomy at the time of transplantation

## IMMUNOLOGIC AND OTHER SPECIAL ASPECTS OF HUMAN RENAL HOMO AND ISOTRANSPLANTS

### Anuria with Recovery

It was shown by Hume and his associates (30) that 4 of *nine human renal homotransplants* were able to go through a prolonged period of anuria following transplantation and then open up diuresis and acquire function adequate to maintain life. No renal homotransplant in experimental animals has ever achieved function once it has become anuric. The reason for this is that destruction of the transplant by immune mechanisms proceeds so rapidly in the experimental animal that the kidney becomes too badly damaged to secrete before it has a chance to recover from the initial anuria The anuria in humans is secondary to the agonal ischemia of the donor kidney and the delay following *death before the blood supply can be reestablished.* It is not inherent in the homotransplanted kidney as was shown in one case by Hume and associates (30) and in another by Michon and associates (20) in which the homotransplant, acquired at operation began to function immediately Nor is it necessarily absent in the isotransplanted kidney *since one of the patients of* Murray Merrill and Harrison observed by the author was anuric for a period of days following transplantation before diuresing

Microscopic evidence of tubular regeneration was noted by Hume and his coworkers (30) in homotransplanted human kidneys Testosterone was used by these authors in all cases because it has been reported to promote tubular repair and for its anticatabolic effects (40–44) Whether this aided the recovery of the transplants or not is problematic but the important point is that the kidney was repairing itself and achieving ever increasing function at a time when transplants in experimental animals would have long ceased to function.

## Function

The function of the four functioning transplants reported by Hume and his associates (30) was about 25 per cent of normal which was sufficient to lower the blood urea and maintain the patient's appetite, hematocrit and general well-being. The function in the recipient was depressed by the prolonged ischemia during and after the terminal period of the donor's life. Nevertheless the longest surviving transplant was capable of responding to water loading and diuretics and weakly to antidiuretics and continued to improve in function until at least the one-hundred and fifty-second day. After it ceased functioning the blood urea nitrogen (BUN) which had fallen from 244 to 34 rapidly rose to 260 and the patient died. No other renal homotransplant experimental or clinical has functioned successfully for this length of time unless an exchange of antigen *in utero* had occurred as with the dizygotic cattle twins. Auto- and isotransplants of course are capable of indefinite survival. In the case reported by Michon and his coworkers (20) the kidney which was obtained from a living donor showed somewhat better function during the brief period of its survival. The isotransplanted kidney in the case reported by Murray and others (33, 35, 38) achieved essentially normal function after the recipient's own kidneys had been removed. It was able to concentrate, dilute, acidify and alkalinize the urine and to respond normally to circumstances producing alterations in G.F.R. and effective renal plasma flow (ERPF). It responded normally to diuretics, water loading and Pitressin. There were abnormalities of sodium excretion which were not, however, attended by any changes in the blood sodium or potassium levels. No abnormalities of function including sodium excretion were seen in the twin isotransplant of Hume and Schilling (30B).

It appears therefore that a transplanted (denervated) human kidney is capable of nearly normal function and that homotransplants can work well if the period of ischemia is as long as one hour and somewhat less well if it is prolonged to $1\frac{1}{2}$ or more hours.

## Hypertension

The experimental work on the effect of renal homotransplants on hypertension has been discussed previously and may be summarized by saying that the kidney will reduce the hypertension if the recipient's own kidneys are removed or excluded from the circulation. The homotransplant itself will not *create* hypertension in the normotensive host unless it is ischemic.

The transplants in the cases reported by Hume's group (30) did not alter the host's hypertension but of course both host kidneys were still present. When the hypertension was mild it did not become worse during the period of function. The transplant in the case reported by Michon and his associates (20) produced a progressive increase in the host's blood pressure although neither host kidney was present and urinary excretion persisted unabated without vascular thrombosis. This finding is not too surprising, however, because patients have been described in whom renal function on one side while nearly normal by usual measurement was still depressed enough to give rise to severe hypertension. It is quite probable that some degree of ischemia existed in this transplant. In the isotransplant in the case of Hume, Schilling and their coworkers (30B) the patient's severe hypertension persisted unabated until his own kidneys were removed. After this it rapidly returned to normal. In the cases of Murray and coworkers (30A) the hypertension came down after the isotransplantation, and before removal of the diseased kidneys. Similar experimental findings have been reported in which homografted kidneys reduced the hypertension of the bilaterally nephrectomized dog (45, 46).

Hypertension should not prove to be a difficult problem, therefore, if permanently successful renal homotransplants can be achieved in man.

## Anemia

The recipient of the kidney transplanted by the French workers (21) was said to have developed a progressive anemia during the 21 days that the kidney functioned. With the anemia associated with uremia there is an almost complete disappearance of erythroblasts in the bone marrow. The anemia of the patient in question was accompanied by 15 per cent erythroblast count of the bone marrow and appeared to be due to hemolysis. It was postulated that antibody formation of the grafted kidney against the host was responsible for the hemolysis. This was shown to occur in dogs when a splenic homograft from an A − dog was placed in an A + dog (Simonsen and Eyquem, quoted by Simonsen (4 )). A positive direct Coombs test developed one week later

with autoagglutinins in the recipient's serum and the development of a severe anemia. The spleen, however, is a more active antibody producer than the kidney and the two dogs were selected to be of different blood types. Also, the development of the anemia coincided with the disintegration of the splenic graft. As mentioned earlier, Muirhead and Groves in 1955 (48) found an associated positive Coombs' test and anemia in dogs undergoing renal homotransplants. The blood types of the dogs were not stated and not all of them showed this finding. Those which did manifested it at the time of homograft degeneration, raising the question as to whether this might be due to a non-immune globulin released by the disintegrated homograft, rather than a specific immune globulin.

It is of interest in this connection that the patient in the case reported by Hume and associates in whom the homograft functioned for nearly six months did not develop an anemia. As a matter of fact, his hematocrit fell to 17 with acute blood loss following surgery and was raised to 27 with transfusions. It skidded to 22 again during the period in which the transplant was anuric and then began a steady upward climb as the transplant function improved and the BUN came down. During the later stages the hematocrit reached values of 41 and 43 without transfusion. The transplant, therefore, appeared to exert a beneficial effect on the anemia secondary to uremia. The biologic incompatibility in this case was apparently far less than it was in the case of the French operators, however, and this may account for the difference in the two.

The development of anemia will presumably not be a problem if the anti-transplant immune response can be eliminated or rendered innocuous. There was a rapid amelioration of the anemia in the uremic patient receiving the isotransplant (34).

## Development of Glomerulonephritis in Transplants

One stumbling block to successful renal homotransplantation in man is the possibility that the transplanted organ might develop the host's disease. Even if the immunity problems of homograft rejection could be eliminated, this might prove an ultimate "Achilles heel." It was shown by Hume and his associates (30) that a renal transplant grafted into a patient with polyarteritis nodosa developed severe acute glomerulonephritis within 38 days, leading to destructive changes which were identical to those that had brought about a complete obliteration of the normal structure of the patient's own kidneys. This kind of response was never seen in the experimental animal. It suggests that patients with polyarteritis nodosa will not become candidates for renal homotransplantation, in the event that this proves immunologically possible, unless some method is also found to prevent the transplant from acquiring the host's disease.

In the experimental work on glomerulonephritis, reference is made to the role of antigen-antibody reactions as possible etiologic factors in this disease. Lange and his coworkers (49) found autoantibodies to human kidney tissue in patients with glomerulonephritis even in the chronic stage. Lippman, Cameron and Campbell (50) found a circulating nephrotoxic substance in patients with glomerulonephritis. It seems theoretically possible, therefore, that a transplant functioning in a patient with chronic glomerulonephritis might fall heir to this disease. Hume and his coworkers (30) homotransplanted kidneys into two patients with chronic glomerulonephritis, and neither transplant showed changes of the disease in spite of the fact that the graft persisted in one of the patients for a period far longer than another transplant had taken to develop glomerulonephritis in the patient with polyarteritis (described above). Six of the 8 isotransplantations reported by Murray and his associates (39A) were performed in patients with chronic or subacute glomerulonephritis. Two of these transplants developed glomerulonephritis and the host eventually died. This would suggest that if the disease is not quiescent auto-antibodies or some other nephrotoxic agent may destroy the transplant. Although it is possible that removal of the diseased kidneys prior to or shortly after transplantation will reduce the incidence of transplant involvement, it is equally possible that this will increase antikidney antibody titre by removing the antigen upon which the antibodies have been fixing. Four of the transplants have not developed the disease and the transplant grafted by Hume and Schilling, also in a patient with glomerulonephritis, has not developed the disease in a period of a year since the transplantation. If this continues to be a problem it may be possible to solve it in identical twins by giving total body irradiation to the host to destroy antibody producing tis-

*and injecting bone marrow from the donor twin* to replace the tissue prior to transplantation.

Chronic pyelonephritis did not pose a problem with respect to transplantation (30, 30A) except that associated with infection.

### *Effect of Hormones*

In several cases reported by Hume and his coworkers (30) ACTH and cortisone were used in an attempt to attenuate the immunizing response. No definite benefit could be ascribed to these substances. Testosterone was also used as described earlier and although no proof is available that it was of any benefit, some tubular regeneration was seen, and the use of testosterone to help protect the transplant is at least theoretically valid.

### Blood Groups and Transfusions

The duration of survival of skin homografts in man does not seem to be intimately related to the comparative blood types of recipient and donor (51). There is some evidence, however, in the work of Hume and associates (30) that the results of renal homotransplants under circumstances of ABO incompatibility are not as satisfactory as when the ABO and Rh types of donor and recipient are identical. An additional human renal homotransplantation was performed in a patient whose blood type was not the same as that of the donor (Hume, Merrill and Harrison, unpublished). The transplant never secreted urine although it was technically successful. If future studies of human renal homografts seem indicated, it would be important to have the donor and recipient of the same blood type. In the most successful of the homotransplantations reported by Hume's group the patients received multiple transfusions apparently without ill effect on the transplant.

### Enclosure in Plastic Bag

In one of the human renal transplantations reported by Hume and his associates (30) the homograft was encased in a sealed plastic bag at the time of transplantation. At autopsy, however, the bag was found to be broken, so that it is difficult to ascribe any role to it other than the destructive changes seen in the glomeruli in that part of the cortex directly subadjacent to the plastic. The reason for attempting to isolate the kidney from the surrounding tissues were discussed under experimental studies. This particu[illegible] f nt functional for the l[illegible]t time.

### *Complement*

Changes in blood complement have been looked for in clinical as well as in experimental studies. Hume and coworkers, (1955) (30) reported no significant change in blood complement level following transplantation in one of their patients. Oeconomos, (1956) (28) also reported no change in complement levels in the work he described.

### Gammaglobulin

In the case reported by Michon and associates (20) and in the one by Ducrot and Antoine (21) there was a progressive increase in gammaglobulin following transplantation, suggesting that there had been an increase in antibody production as a consequence of the transplant. This very interesting observation is congruous with the rapid rejection of the transplant seen in this case.

### Antibody Production in Chronic Uremia

Patients with Bright's disease show hypoproteinemia and exhaustion of reserve protein, due partly to decreased protein intake and urinary loss, but also partly to depressed formation of serum protein. In the nephrotic syndrome the hypoproteinemia is characterized by very low levels of gammaglobulin in spite of the reversed A-G ratio. There is some evidence that under these circumstances antibody formation and resistance to infection are decreased (52), although this has been disputed by others (53-55). Whether or not a decrease in plasma proteins a a consequence of starvation produces an alteration in the ability to react to bacterial antigens is not necessarily germaine to the present discussion. The alteration in plasma proteins in uremia is not invariably equivalent to that in starvation and in fact has been shown not to be in some instances. In addition, the ability to react to bacterial antigens cannot always be equated with the effectiveness of an antibody response to homograft. Furthermore alterations in some other parameter of the complex immunity response against homografts may occur in long standing uremia.

*It is possible that a subnormal antibody production in chronic uremia, whether due to change in plasma protein or not, may have contributed to the prolonged survival of some homograft* in the cases reported by Hume and associates (30). The rapid destruction of the homograft in the case reported by Michon's group (20) may have been related to the fact that the patient was a

healthy young boy with acute uremia but with presumably normal mechanisms of immunity.

Dammin, Couch and Murray (56) have reported prolonged survival of skin grafts in uremic patients lending credence to this theory.

Attempts to produce attenuation of the immunity response in dogs by the creation of chronic uremia failed (57) but the duration of the uremia may well have been too short to be effective.

**Intimal Changes in Vessels of Renal Homotransplants**

In the section on experimental renal homografts it was emphasized that changes in the endothelial cells of transplant blood vessels are an integral aspect of the histologic alterations occurring in primary renal homografts in dogs although apparently not in goats. Carrel (58, 59) also reported marked calcification of the host's blood vessels, without involvement of those of the transplant, following renal homotransplantation in one cat.

Hume and his associates (30) reported one human homotransplant functioning in the host for a period of nearly six months, which at postmortem examination showed marked intimal proliferation of many of the intrinsic renal vessels (figs. 208 and 209). The inner portion of the intima showed dense sclerosis, and the outer layer was filled with lipid laden macrophages. The recipient had continued to have rather severe hypertension while the donor had had mitral and aortic stenosis associated with a low blood pressure. Whether the intimal changes were a reflection of the sudden alteration in dynamics to which the homograft was subjected or whether they represented a counterpart of the endothelial changes observed in dog homografts is a matter for speculation. Darmady, Dempster and Stranack (29) reported endothelial changes similar to those seen in dogs in the human kidney homotransplanted by Michon and coworkers (20). In this case there was a differentiation of endothelial cells into plasma cells, and the changes were not like those in the case reported by Hume and associates nor like those occurring in other human renal homografts in cases reported by the latter authors.

It is possible that endothelial differentiation of the type seen in dog primary renal homografts may occasionally occur in human tissue when transplant rejection is of a rather severe acute nature. In the French case the transplant was rejected rapidly—in 21 days—in spite of the fact that it was from mother to son. In the case of the transplant reported by Hume's group the slower rejection, which certainly represented either a greater degree of compatibility or a reduced ability of the host to form antibodies, qualitatively showed different endothelial changes. It may be that there is a gradation of endothelial changes—ranging, perhaps, from the explosive blood vessel dissolution without endothelial proliferation characteristic of secondary transplants, to the endothelial differentiation of dog primary homotransplants to moderately rapid endothelial thickening as in the case of Hume's group, to the slower aging changes seen in the "normal" individual, the latter also perhaps traceable to "immunity" mechanisms. The final answer to this problem awaits further experimental evidence on the nature of the changes.

**Donor Sex**

Recent reports have suggested that the sex of the donor may be of importance to homograft survival, female tissues being tolerated better than male, perhaps because of antigens associated with the Y chromosome in the male. The rapidly rejected kidney in the case reported by Michon and associates came from a female donor (the mother) while the kidney surviving for the second longest period in the series reported by Hume and associates came from a male donor. The longest survival time in this series was shown by a female kidney transplanted into a male, and two other kidney transplants of prolonged survival were from female donors. Mechanical difficulties, or difficulties in collection, make an evaluation of the other female and male donors unsatisfactory except for the kidney transplanted into the patient with periarteritis nodosa, which developed glomerulonephritis and which was from a male into a female.

The human cases of renal transplantation did not, therefore, show any conclusive relationship between the sex of the donor and the survival of the transplant. In the section on experimental studies written before the importance of the sex of the donor was known it was pointed out that in cats the longest transplant survivors in Carrel's experience were both in female-to-female pairs. The female-to-male pairs were complicated by abscesses, but the male-to-male transplants and male-to-female transplants were both short-

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lived. The effect of sex is difficult to evaluate in Simonsen's studies because many of the animals underwent additional procedures and accurate collections were not always possible. All of the pairs in Dempster's (60) series were females. His seven transplants in dogs in which one host kidney had been removed functioned for an average of 3.7 days (range 1 to 8 days). The 53 primary homotransplants performed by Hume and Egdahl (61) and by Egdahl and Hume (62) in dogs in which one host kidney had been removed, involved only male-to-male pairs and functioned for an average of 5.0 days (range 2 to 14 days). It seems therefore that the sex of the donor is not important to renal homograft survival in the dog.

## FACTORS RESPONSIBLE FOR ARREST OF FUNCTION OF RENAL TRANSPLANTS

Dempster (63) has discussed the possible causes of the arrest of renal homotransplant secretion. Plasma cell proliferation within the transplant has certainly been ruled out as the cause of functional arrest, because arrest occurs in the absence of plasma cell proliferation—in primary transplants which are irradiated or obtained from cortisone treated hosts, or in many secondary transplants. The increased size of the transplant, vascular stretch, and increased intrarenal pressure also seem to be eliminated as causes of the arrest.

Dempster mentions the possibility that antibody production by the tubule cell may bring about its own destruction and supports this by the results obtained in retransplanted kidneys, which function for a time and then cease functioning. It may be that under these circumstances continued plasma cell proliferation contributes to the anuria of the retransplanted kidney, but it is difficult to believe that the tubule cell can manufacture antibody in significant amounts. If this were the case, the animal given total body irradiation sufficient to destroy the reticuloendothelial system would not be able to accept bone marrow homotransplants because the functioning non-reticuloendothelial cells in the body would produce antibody and destroy the transplant. In fact, however, such an animal can accept a bone marrow transplant.

Dempster also raises the possibility that foreign protein circulating through the transplant may damage the tubule cell and produce anuria. This seems unlikely in view of the results obtained by Egdahl and Hume (64) which indicate that cross-circulation appears to protect the renal transplant exposed to host antigen, and in view of the retransplantation experiments of Dempster himself in which the anuria occurs in the original donor at a time when the transplant is not exposed to the host's antigen.

Dempster tends to equate the changes in renal homografts to the phenomenon of Masugi nephritis, and to believe that the location of the renal antigens and the site of antibody fixation are in the basement membrane of the tuft. Simonsen regards the changes in the renal homograft as an experimentally produced periarteritis nodosa. Neither of these concepts appears to be consonant with the experimental and clinical observations now available. The renal changes in Masugi nephritis and in periarteritis nodosa are primarily glomerular. In the human renal homotransplantations performed by Hume and associates (30) in the patient with periarteritis nodosa the homograft developed glomerulonephritis with massive proliferation of the epithelium of the parietal layer of Bowman's capsule and the adjacent capillary loops, leading to crescent formation. These changes have not been seen in any other renal homotransplant, human or experimental. It seems evident, therefore, that there are fundamental differences between the site of fixation of the antibodies in the kidneys in such diseases as periarteritis nodosa (a hypersensitivity phenomenon), human glomerulonephritis (if indeed antikidney autoantibodies do exist in this disease), Masugi nephritis (a heterologous antibody), and homotransplant rejection. It is quite possible that the homotransplant antigen (presumably a desoxyribonucleic acid located in the nucleus) is not located in the glomerular basement membrane as Dempster supposes, but is located instead in the nuclei of the tubule cells and perhaps also in lesser amounts in the endothelial cells of the small cortical vessels.

Dempster argues that the individual specific antigen is common to all tissues of any given organism and implies that the location of the antigen within the kidney is uniform so that the glomerular tufts should show signs of change if the destruction of the primary transplant is due to an antibody-antigen reaction. His own experiments, however, have shown that the endothelial changes and the plasma cell proliferation of the interstitial reticular cells are of transplant origin,

and thus the only changes in the primary transplant which could be ascribed to host antibody are the tubular ones. If it is postulated therefore, that the greatest concentrations of individual specific antigens are located in the tubule cell nuclei the findings in primary transplants, primary irradiated transplants, retransplants, secondary transplants and cross-circulation could be explained as follows

1) In primary transplants the host antibody builds up slowly fixing on the tubule cell and gradually producing tubular necrosis and anuria. In man the build-up of antibody is very slow as in the case reported by Hume and associates and it may also fix on the small amounts of antigen located in the endothelium of the intrarenal interstitial vessels, stimulating these cells to proliferation. The transplant itself reacts against host antigens by the transformation of endothelial cells into plasma cells in the dog, but not in the goat and only rarely in man. The interstitial reticular cells react against the host and proliferate. Vascular spasm of cortical vessels may also be a manifestation of transplant reaction against the host.

2) Primary irradiated transplants show the tubular changes brought about by host antibody fixing on tubular antigens, but do not show the changes which are a consequence of transplant R E reaction against the host.

3) Retransplants replaced before host antibody can fix upon the transplant show no changes. Some retransplants returned after more than 48 hours in the host ultimately become anuric, as host antibody fixed to the tubular antigens in weak concentrations finally produces destruction leading to anuria. The persistence of host antigen in the transplant, or more likely continuation of the R E differentiation brought about by it, leads to R E proliferation by the retransplanted kidney After all in Simonsen s experiments the only retransplants showing continued plasma cell proliferation were those that already showed it at the time of retransplantation. This point was not established in Dempster's series.

4) Secondary transplants show an explosive tubulorrhexis due to rapid destruction by increased concentrations of antibody and may also show blood vessel damage and hemorrhage as tissues containing smaller amounts of antigen are also affected.

5) Cross-circulation at the time of transplantation protects against destruction of the secondary transplant by neutralizing the host antibodies with circulating antigens.

The rapid destruction of the secondary transplant makes it more difficult to observe the sequential changes of rejection. The slower changes in the primary transplant, particularly in man, seem to be consistent with a location of the renal individual specific antigen principally in the tubule cells and not, as postulated by Dempster in the basement membrane of Bowman s capsule. This latter antigen is important to the development of Masugi nephritis and the glomerulonephritis of polyarteritis nodosa but probably not to renal homotransplant rejection

Clearly there are many areas in which the immunity responses to renal homografts should be clarified. Some of these share the problems of all homografts, and must be approached at a cellular level and not through additional renal homotransplants Others however are unique to organ transplants in general and renal transplants in particular and can be clarified by experiments designed to show the time needed for antibody fixing the location of the individual specific antigens, the mechanism of anuria and many other facets of this interesting problem.*

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# PART X

# *Peritoneum*

CHAPTER 15

# Transplantation of Peritoneum

ABDOL H ISLAMI

### Introduction

Surprisingly little if any mention is made of peritoneal tissue in general textbooks on anatomy and physiology suggesting a limited knowledge on the part of most medical students and practicing surgeons of the structure and function of peritoneum. The omentum however is well known for its important role in protecting the peritoneal cavity Whenever a lesion of one of the intraperitoneal organs or of their adjacent reflections threatens to involve the serous cavity the omentum adheres to the particular organ and forms a barrier against the process. Freeman, in 1916, made the observation that the omentum seems to seek out and attach itself to raw or inflamed surfaces, wrapping itself around them in such a way as to afford the maximum of protection. Freeman wrote 'Nature often uses the omentum within the peritoneal cavity much as a surgeon employs adhesive plaster or a dressing externally—for temporary protection only"

In the latter part of the nineteenth century and the early part of the present century extensive literature on the subject indicates that surgeons engaged in experimental work were using peritoneum for transplantation. Although aseptic measures and prevention of infection were not what they are today the results obtained proved to be remarkably favorable. At the present time of great surgical advancement, with improved operative technique and the use of antibiotics, surgeons could well take greater advantage of the possibilities of peritoneal tissue, since it is available for transplanation in considerable quantities within the human body To be sure, in recent decades the once sporadic interest of surgeons has changed to genuine and sustained concern about these possibilities.

## ORIGIN AND DEVELOPMENT OF PERITONEAL TISSUE

Peritoneum, lining the abdominal cavity is the largest serous membrane in the body that is derived from mesenchyme (mesoderm) Its surface area is approximately equivalent to that of the skin. The peritoneum is not epithelial in character or in function it differs from epithelium in its ability to repair itself

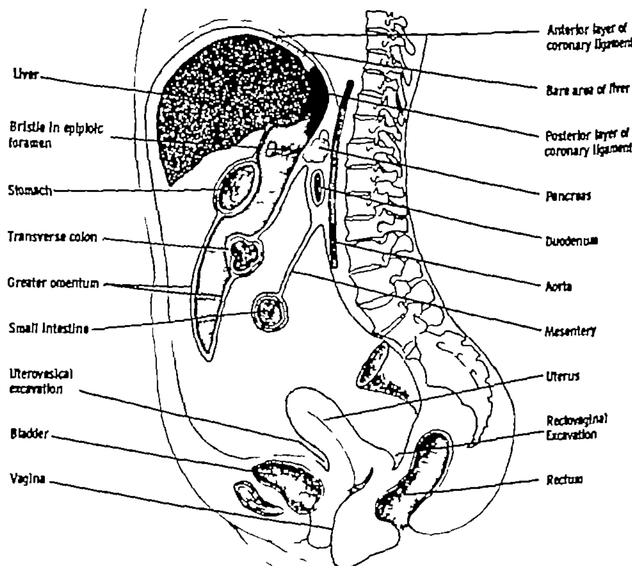
In the embryo the mesoderm splits into two layers a dorsal layer called the somatopleure and a ventral layer called the splanchnopleure. Between the two layers the coelom, or body cavity is formed. This cavity is ultimately divided into pericardial, pleural and peritoneal parts. The pleural and peritoneal cavities are embryologically the same, being separated by the diaphragm. From the stomach to the rectum the gut is attached to the notochord by a thin layer of mesoderm, the dorsal mesogastrum, from which the common mesentery is subsequently developed. There is also a ventral or anterior mesentery attaching the ventral aspect of the gut to the anterior abdominal wall, the caudal portion of which forms the ventral mesogastrum.

The gastrointestinal tube grows disproportionately its rate of growth being more rapid than that of the embryonic cavity At the same time differentiation occurs producing segments which become the stomach and the small and large intestine. In the final stage of development the segments of the tube become fixed through permanent fusion of certain mesenteral supports with apposed areas of parietal peritoneum.

## DISTRIBUTION OF PERITONEUM

The presence of peritoneal tissue in the human body is limited to the abdominal cavity. In the male the peritoneum forms a closed sac (the greater bursa) with a diverticulum of the same tissue forming the lesser sac or bursa. These two cavities communicate with each other by means of a tube of peritoneum (the foramen of Winslow). In the female the free end of the Fallopian tubes opens into the peritoneal cavity. Surgically the peritoneal cavity can be divided into an upper or supracolic space and a lower or infracolic space. The supracolic space is located between the diaphragm above and the superior border of the mesocolon below. The infracolic space extends from the inferior aspect of the mesocolon to the rim of the true pelvis; finally the lowest compartment or true pelvic cavity is located between the rim of the pelvis below and the pelvic diaphragm above.

The supracolic space is divided by the liver into supra- and infrahepatic portions. The suprahepatic space is divided into right and left halves by the falciform ligament. The right suprahepatic space is further subdivided by the triangular ligament of the right hepatic lobe into anterior and posterior segments. Below the liver and to the right of the falciform ligament




Vertical section showing disposition of the peritoneum.

Fig. 212

is a single large space (Morrison's space). Thus the right side above the colon can be divided into the following peritoneal segments: 1) the right anterosuperior intraperitoneal space, 2) the right posterosuperior intraperitoneal space, 3) the right inferior intraperitoneal space and 4) the right extraperitoneal space (the bare area of the liver). To the left of the falciform ligament there is a space anterior to the left triangular ligament. This latter ligament extends along the posterior border of the left lobe of the liver and separates the superior from the inferior surfaces. Thus there is only one space to the left of the falciform ligament in the front, namely, the left superior intraperitoneal space. Below the liver on the left side, there are two spaces, viz. a posterior space (actually the lesser bursa) and an anterior space in front of the lesser omentum and stomach. Hence the spaces on the left side above the colon are: 1) the left superior intraperitoneal space, 2) the left anteroinferior intraperitoneal space, 3) the left posteroinferior intraperitoneal space, and 4) the left extraperitoneal space. These subdivisional areas are important because they become complicated areas of infection.

The infracolic space is divided into two somewhat triangular areas by the root of the mesentery of the small bowel. The line of the mesenteric root extends from the region of the second lumbar vertebra on the left of the midline to the right sacroiliac joint. Lateral to each triangle a gutter can be delineated by which the upper abdominal areas communicate with the true pelvis. These are the right and left paracolic gutters.

## HISTOLOGIC STRUCTURE OF PERITONEUM

Peritoneum is a smooth serous membrane lined with a layer of flattened epithelial cells known as mesothelium. These cells are attached to each other laterally by delicate cytoplasmic arms that extend into an intercellular cement substance. The mesothelial cells are mesenchymal in origin and thus have the potentiality of differentiating into various specialized types of adult connective tissue. They rest on a basement membrane which is approximately 8 to 10 microns thick where the peritoneum covers the stomach but is much thinner elsewhere. Beneath the basement membrane is a layer of connective tissue, which consists of more or less parallel bundles of collagen over a feltwork of elastic fibers that covers a latticework of collagen fibrils. This fibrous layer of peritoneum often contains fat. Smooth muscle occurs frequently in various peritoneal folds. The peritoneum is sparsely supplied by blood vessels and lymphatic vessels and contains a few nerve fibers. Peritoneum lines the peritoneal cavity, overlies the visceral organs, and surrounds the intestines completely covering the appendices epiploicae—the small fatty lumps attached to the large intestine. The portion of the peritoneum which covers the wall of the abdominal cavity is called the parietal peritoneum. The visceral peritoneum covers the abdominal organs and bowel. In the parietal peritoneum the fibrous layer is distinct, loose and movable, whereas in the visceral peritoneum it is firmly fixed to the organs and difficult to identify as a separate layer.

### Mesentery

The parietal and visceral peritoneum are connected with each other by the mesenteries or ligaments. These function as suspensories for the organs and gut. The mesentery tissue is composed of a loose network of collagenous and elastic fibers with varying numbers of fat cells, scatter fibroblasts, macrophages and mast cells. This areolar network carries blood vessels, lymphatic vessels and nerves and is covered with peritoneum on both surfaces.

### Omentum

The omentum has a structure that is similar to the mesentery, with its surfaces likewise covered with peritoneum. It has numerous fenestrations and is thus reduced to a fine lace-like network formed by collagenous bundles covered with mesothelial cells. The fenestrated areas, having few or no vessels, are free of tissue and permit communication of fluid in cells from one side of the serosal membrane to the other. Macrophages are numerous throughout the omentum and undifferentiated cells are present along the paths of the blood vessels. The macrophages tend to accumulate in dense masses, which are often arranged along the blood vessels. These are visible as small or large, round or oval patches, formerly described as milk spots, which are very characteristic in the omentum of the rabbit. The macrophages undoubtedly play a very important part in the defense mechanism of the peritoneal

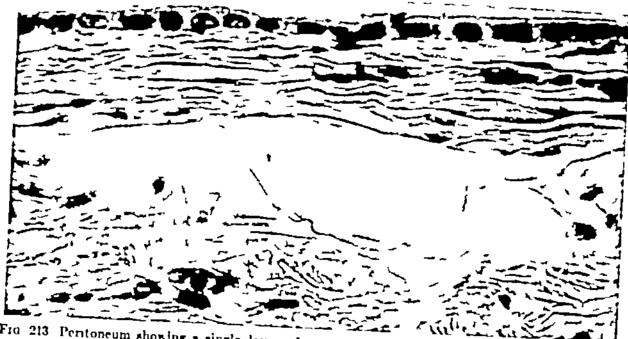


FIG 213 Peritoneum showing a single layer of mesothelial cells covering a fibrous stroma ×600



FIG 214 Peritoneum showing a flattening of its single layer of mesothelial cell ×600

cavity because of their functional characteristic of phagocytosis Also present are many lymphocytes and plasma cells and occasionally eosinophil leukocytes and mast cell

## Significance of Peritoneal Structure in Transplantation

The advantages of the protective property of peritoneum have been recognized since the mid nineteenth century It was then proposed that omentum be systematically used and in a general way the peritoneum itself as a valuable aid in suturing and securing hemostasis of wound of the liver spleen, vessel and other organs The properties of peritoneum that favor its use for transplantation are lack of a vascular system of its own ability of the serosal surface to unite quickly with surrounding tissue and the security

of its nutrition through permeation in the first few hours after transplantation.

The lack of a vascular system in the peritoneum refers strictly speaking, to the fact that it is not a self-dependent tissue. In reality it is abundantly supplied with a dense system of blood capillaries and lymphatic vessels ascending on the peritoneum from its base and ramifying as well in the subserosa as in the serosa itself. Despite this, the peritoneum usually does not bleed after it is cut. Furthermore, individual nerve endings are located in the subserosa under the cellular membrane.

Portions of parietal and visceral peritoneum including the protruding part of the parietal peritoneum of a hernial sac and portions of the peritoneal surfaces of omentum mesenteries, ligaments and appendices epiploicae have all been used for purposes of transplantation. The elastic fibers of the peritoneum condense near the epithelial surface to a so-called borderline, which is the important landmark in transplantation operations. These elastic fibers are not nearly as copious in the omentum as in the other tissues covered with peritoneum

The peritoneal surfaces are constantly being moistened by a small amount of protein-containing fluid, called the liquor peritonei, which allows the organs to glide easily against each other It contains macrophages derived from the omentum mesothelial cells desquamated from peritoneal surfaces, lymphocytes, a few tissue mast cells, and some neutrophilic leukocytes. The liquor is readily increased by mechanical, chemical or bacterial irritation. As an inflammatory exudate, it is rich in fibrin and cells, and thus is the cause of adhesions.

The contents of the abdominal cavity are normally neutral or weakly alkaline. It is this property which probably accounts for the natural prevention of fusion between the parietal peritoneum and the visceral peritoneum. On the other hand, it is the acid reaction associated with an inflammatory condition of the peritoneum which sets up the irritation between the parietal peritoneum and the visceral peritoneum and causes fusion of the two tissues.

This tendency of the peritoneum to form a fibrinous exudate is an important characteristic of all serous membranes. It is of value in the healing of wounds and in many types of peritoneal transplantation. However when gliding surfaces are required the presence of a fibrinous exudate limits the possibilities of this tissue as transplants and is undoubtedly the factor accounting for the use of peritoneum less frequently than other tissues for transplantation (1–3)

## FUNCTIONS OF PERITONEUM

*Lubrication.* Peritoneum provides a smooth moist surface which insures that the various organs of the abdominal cavity glide easily and without damage over each other The serous exudate of the peritoneal cavity (derived from the blood stream) lubricates this process of smooth contact.

*Support.* Peritoneal tissue is also characterized by a certain tensile strength which serves to supplement the ligaments and muscles in supporting the viscera in the abdominal cavity

*Protection.* The entire abdominal cavity containing the viscera is enclosed in the parietal peritoneum and each viscus itself is likewise totally or partially covered with peritoneal tissue. Peritoneum thus acts as a protective tissue around the abdominal organs, each organ being prevented from adhering to adjacent ones. Moreover the omentum acts to some extent in circumscribing any disease process by adhering to the involved organ sealing off perforations and forming a capsule to wall off the spread of infection.

*Sensibility* The free nerve endings of somatic nerves that branch in the subperitoneal tissue are pain receptors Exertion of pressure on the parietal peritoneum, therefore, incites pain which is fairly well localized and associated with cutaneous hyperalgesia it also produces contraction of the abdominal wall muscles over the stimulated area. The same sensibility is present in the peritoneum lining the diaphragm as in the diaphragmatic pleura, i.e. the peripheral part of the peritoneum is innervated by the intercostal nerves and the central part by a phrenic nerve. Pain arising in the periphery is referred to the base of the thorax and the upper part of the abdominal wall on the affected side over an area covered by the dermatomes of the seventh to the twelfth thoracic roots. Pain arising in the central area is referred to the shoulder and lower part of the neck. The omentum is apparently relatively insensitive whereas the peritoneum covering the duodenum and pancreas, on the contrary has intense sensibility Traction on the mesentery and stimulation of the mesenteric blood vessels or perivascular tissue produce

intense pain referred to the epigastrium and umbilical region (4).

## REVIEW OF LITERATURE ON PERITONEAL GRAFTS

The method of transplanting peritoneum has been used for a long period of time. Clinical experience showed that in pathologic changes in the abdominal cavity the omentum glides to the diseased part to cover and to protect it, as previously mentioned, adhering to it, closing perforations and encapsulating pus formation, thus tending to prevent generalized peritonitis. Omentum therefore was used to cover perforations, but at first only as pedicled transplants (5).

In 1826 Jobert closed stomach wounds which he had inflicted on dogs by inserting a pedicled portion of the omentum into the wound. Clinically the parts were used to close gastric and duodenal ulcers after the method of Braun and Bennet, so that no one individual may be called the inventor of the method.

The healing process occurs by shrinking of the transplanted omentum. It was especially Tietze and Enderlen who devised the method of transplanting omentum. They covered defects in the stomach, intestines, gallbladder and urinary bladder with pedicled omentum and stated that shrinkage of the omentum and contraction of the muscularis mucosae occurred. They also demonstrated that the ability of the mucosa to regenerate by spreading from the edges to the center of the defect shortly produced a complete mucosal covering over the defect. Mandl and Cara from the Clinic of Eiselberg tried to cover parts of the intestine which were bare of peritoneum and also reenforce sutures in these organs but results of clinical experience were not reported. Goepel and Hoerhammer used a serosa muscularis cuff with good results. Wide use was made of the peritoneal transplant in covering peritoneal defects after serosa visceralis was removed when there were adhesion, and also for covering defects of the liver bed in cholecystectomy (von Langenbeck, Kehr, Witzel, Rohde). In the last instance peritoneum was also employed for the purpose of controlling bleeding from the liver bed, from wounds of the parenchymatous organs of the peritoneal cavity (liver, spleen, pancreas) and from wounds of the kidneys (5).

### Early Experimental Work on Animals

The earliest observations leading to the use of peritoneal tissue for grafting were concerned with its adhesive quality. Hegar and Hamilton in 1881, Dombrowski in 1888 and Thompson in 1891 showed how rapidly adhesions are formed by omentum (epiploon) with foreign bodies such as gauze and fragments of sponge left in the peritoneal cavity. The foreign body was truly grafted and confined by the serous adhesion. Likewise, Jalaguieret and Mauclaire in 1897 left similar foreign bodies in the peritoneal cavity of rabbits and dogs and observed that omentum was adhered to the sponge. Kaumetroff and Pensky applied iodoformed gauze on the wound resulting from partial resection of the liver and noted that frequently the omentum becomes adhered to the wound while producing hemostasis. Auvray stressed the grafting of autogenous omentum on the edge of a hepatic section (6).

In 1888 Senn (7) was the first to advocate the use of free omental graft. Experimentally he found that the grafts adhered firmly to the surfaces to which they were applied, and they had become vascularized in a very short time. His results observed from 30 hours to 8 days were better with grafts placed upon scarified intestine.

Lanz (8) in 1892 unsuccessfully transplanted pieces of peritoneum into defects of the skin surface and mucous membrane in experiments with animals. In experiments on dogs Cornil and Cornat (9) in 1898 demonstrated the repair of wounds of the common bile ducts by means of omentum, which adhered to the margins of the wound.

It appears that the first successful attempt to use peritoneal tissue for grafting was made by Tietze of the Surgical Clinic of Breslau in 1899 (10). He removed an autograft from the anterior wall of the stomach and replaced it *in situ* in dogs. He presented a drawing of the healed-in omentum, and an illustration of the omentum in the defect taken 8 weeks after grafting. Following an inflammatory reaction in the implanted omental graft the superficial epithelia of the implant disappeared and newly formed epithelium began to develop in a simple layer of cylindrical cell.

In his thesis *Méthode des greffes péritonéales* in 1901, Loewy (11) describes the application of

autogenous or homogenous omentum as a graft sutured to the surface of the lesion to secure hemostasis in sectioned liver The experiments carried out on guinea pig, rabbit, dog and monkey, even with infected wounds of the liver gave identical results. In two months the omentum was impacted against hepatic tissue. It became adherent to the liver either directly or intermedially by fibrinous leukocytic exudate. Starting from the sixteenth day the omentum was transformed into thick fibrous tissue containing many blood vessels filled with blood. Moderate necrosis of the hepatic cells along the suture lines disappeared rapidly and after the sixteenth day none was evident.

In a resected stomach of the rabbit autogenous omentum was spread over the suture line and sutured to the stomach. Loewy also reports on resections of the intestine and other portions of the digestive tract in animals in which sectioned omentum was sutured over an end-to-end anastomosis. One and one-half hours after the operation the bleeding ceased completely

In the first hours following operation autopsy of the animal showed that the peritoneum was already becoming adherent, and there was also thickening of the wall of the stomach in the region of anastomosis. After a month the omentum completely disappeared. In the process of cicatrization Loewy noted the rapidity of healing of the mucosa, the formation of giant cells around the foreign bodies included in the different layers of the organ and the slight leukocytic reaction of the chromatized interstitial tissue.

Writing in 1902, Senn (12) referred to experimental work on dogs carried out 12 years previously in which omental grafting prevented adhesions, furnishing the line of suture with a band of living tissue that would guard against extravasation and the formation of parietal and visceral adhesions. He stated that in all these experiments on dogs the omental grafts retained their viability and in a few hours became firmly adherent to the intestinal surface with which they had been brought in contact. Where scarification was done the adhesions were firmer and vascularization more advanced. Autopsy examination demonstrated that the firmness of adhesions and the degree of vascularization were in direct proportion to the extent of the traumatic irritation of the peritoneum. Senn foresaw that omental grafting could not fail to become an established procedure in many abdominal operations

Senn (13) in 1903 reported on transplantation of omentum over intestinal defects in dogs. The omentum being thinner in the dog than in man, the unfavorable results in his opinion, were not a criterion of the future value of human omental transplantation.

Carrel (14) in 1906 reported on using many substances among them peritoneum to secure patches into the walls of blood vessels in a cat. The animal lived as long as 7 months postoperatively

Loewy (15) (1903) experimenting with animals, made a gastric resection and applied an omental flap When the animal was sacrificed in the first hours after operation there was adhesion between the flap and the subjacent peritoneum He described the microscopic process. When an omental wad had been placed on a hepatic laceration and the animal sacrificed in one or two hours, the bleeding was completely controlled, and the omental tampon projected on the surface of the liver

Springer (16) (1906) experimented mainly on the small intestines in dogs, using free omental flaps and observed extensive formation of adhesions and healing of the omental covering He also transplanted free omental pieces into the stomach and liver After anastomosis of the small intestine in a dog, the jointure line was covered with an omental flap On the third day the omental flap had adhered to the surface but was raised over the anastomosis. A suture dehiscence with necrosis was observed. Springer thought that no special advantages were gained from the use of free omental flaps except in isolated cases and saw no advantages in practical surgery

Ammann (17) (1906) recommended the use of omental grafts for defects of the bowel From experimental evidence he was convinced that almost any part of the peritoneum may be used in covering peritoneal defects. In experiments with animals Girgolaff (18) (1906) confirmed the hemostatic efficiency of free transplanted autoplastic omental flaps but he could not prove the direct adhesion of the omentum to the liver He stated that there always was fibrinous exudation or blood between the omentum and the liver The flaps healed and showed the formation of capillaries after 24 hours. He explained the control

of the bleeding by a tamponic effect of the omentum.

Using rabbits cats and dogs, Boljarski (19) (1910) removed sections of liver and pressed free flaps of omentum on the wounds as tampons for control of bleeding or spread and pressed omental flaps as covering over raw surfaces extending them over the edges of the wound. His experimental results were favorable and he believed that omental plasty is clinically applicable for controlling bleeding in puncture wounds of the liver and reported cases to support his contention

Wilkie (20) in 1911 reported on several experiments to test the value of grafting isolated portions of omentum to reenforce suture lines In his opinion his results fully confirmed that this practice has the unavoidable disadvantage that it almost invariably leads to extensive peritoneal adhesions to surrounding viscera. He did think however that application of a corner of the normal attached omentum to a doubtful suture line the duodenal stump in a pylorectomy for example was a method of undoubted value.

Negri (21) also in 1911 used a free transplanted flap from the parietal peritoneum in experiments on animals and after control of the bleeding by manual compression of the liver he sutured flaps of the parietal peritoneum into the defect to the underlying muscle under tension. The results were good.

Comprehensive experimental studies with peritoneal grafts, both fresh and preserved were made by Kolaczek in 1912. He obtained the best results with fresh homografts (22) He inserted peritoneum into a capsule of the knee joint in animals and noted no adhesions up to 47 days postoperatively The function of the joint was normal in all instances. It was found that in the joint the peritoneum serves only as a skeleton for the newly formed capsule After two weeks the transplanted peritoneum was not present on the surface of the joint but had been pushed into the deeper layers (23)

In 1913 Colanitski (24) successfully employed peritoneum as suture material in animals He used auto- homo- and heteroplastic peritoneum which remained for at least three months without change Primary healing of the wounds always occurred. In several experiments Jacquin (25) in the same year covered large defects of the liver up to one-fourth of the organ with omentum and obtained complete hemostasis

He noted that within two months the omentum was transformed into fibrous (connective) tissue and there was firm adhesion with the surrounding region.

Sweet Chaney and Wilson (26) (1915) carried out experiments on dogs to ascertain the effect of covering the operative area with an attached portion of omentum. Two end-to-end anastomoses were performed on two different loops of intestine in a number of dogs the first being left free without covering the second being covered with the free border of the omentum. The omentum was carefully wrapped over the site of the anastomosis and held in position by silk stitches. They also experimented on dogs to observe the effect of free omental and mesenteric grafts for the prevention of adhesions

Experiments on animals were carried out by Carl B Davis (27) in 1917 with free transplantation of omentum both subcutaneously and within the abdominal cavity He concluded that omentum transplanted freely beneath the skin in masses one inch in diameter maintains the greater part of its bulk. His microscopic examination of the grafts demonstrated viability of the cells. It seemed to be of little consequence whether the graft was one-half inch or three inches in diameter Also in 1917 Lawrora (28) concluded from his experiments in which he removed the bone marrow and implanted omentum into the cavities that the omentum was replaced by granulation tissue and finally bone marrow The defect of the bone was largely replaced by regeneration from the periosteum.

In experiments with free transplanted pieces of peritoneum in animals Keysser (29) (1918) proved the value of homoplastic hernial sacs In two attempts the transplant of hernial sac seemed to heal but after about two weeks failed to take In one experiment acute gangrene of the transplanted tissue resulted.

Peet and Finton (30) (1919) studied omental grafts histologically and determined that in dogs the grafts survived unchanged for at least 6 months They recommended that the thinner the graft used the better was the result.

In his excellent work *Die Freien Transplantationen* Lexer (31) (1924) reported on various experiments with peritoneum in animals Circular defects of the tendon sheath in dogs were covered with autoplastic peritoneum with the serosa facing the tendon. In all animals sacrificed after varying period of time union occurred between

the transplanted tissue and the tendon, dense adhesions being observed after 76 days. Microscopically the peritoneum showed partial degeneration and some proliferation of the connective tissue cells but especially proliferation of the connective tissue of the surrounding areas, into which blood vessels were penetrating after 11 days capillaries filled with blood were observed within the transplanted tissue. The growth of capillaries and connective tissue cells between the transplanted peritoneum and tendon were noted.

When defects of the joint capsule were covered with parietal peritoneum after application of cartilage in dogs and rabbits, dense adhesions were noted between the transplanted tissue and the surfaces of the joints. Attempts to cover circular defects of the abdominal aorta with fresh parietal peritoneum in dogs were unsuccessful.

Portions of the anterior pericardium were removed by Lexer in dogs cats and rabbits, and peritoneum was transplanted into the defects without loss of pericardial fluid. The endothelium faced the pericardial cavity and a normal amount of pericardial fluid was present within the pericardium. In sacrificed animals from 82 to 74 days postoperatively there were extensive firm adhesions between the transplanted peritoneum and the epicardium. Histologically only a few cells of the transplanted tissue survived and participated in covering the defect.

Defects in the parietal pleura of animals were similarly covered with transplanted peritoneum. Partial resections of the lungs were also carried out and the bleeding surfaces were covered with peritoneal transplants after suturing of the larger blood vessels and branches of the bronchi. At autopsy there were always fibrinous adhesions, and in later stages firm adhesions of the transplanted tissue and the surrounding parts of the pleura with the opposite parts of the lungs. In contrast to the transplantations on the pericardium there was much less necrosis of the transplanted tissue Parenchymatous bleeding from the lungs could not be controlled and a hemorrhagic exudate into the pleura later became purulent.

Defects made in the dura of dogs were covered with parietal peritoneum. At autopsy adhesions of the transplanted peritoneum and the surrounding dura with the pia or brain were present. Regeneration in Lexer's opinion, occurs partly through surviving cells of the transplanted tissue but in a much greater degree through invading connective tissue from surrounding areas

Bothe (32) (1929) severed thick and thin grafts of various sizes free from the border of the greater omentum in dogs and sutured them to underlying tissue over an excised gastric ulcer, to cover intestinal perforations, and artificial serosal defects in the spleen and liver accompanied by severe hemorrhage. Macroscopic observations and histologic studies were made of the changes which occurred in the grafts from 3 days to 4½ months after transplantation. He considered thin grafts preferable, and found that freed omental grafts unite far more satisfactorily when the peritoneum has been denuded. Union is complete. Absorption occurs and there is almost complete absorption of the thin graft at 4½ months.

Lovering (33) (1935) found by comparative measurement that the tensile strength of the peritoneum is considerable, but not nearly so good as that of fascial structures. Experimenting with dogs he demonstrated that the peritoneum has sufficient tensile strength so that it may be utilized in the repair of certain inguinal hernias. The experiments also showed that peritoneum will unite with fascia, and with the perimysium of muscle in a manner similar to that of the fascial suture. Levering suggested that the hernial sac be used in operations where reenforcement of the abdominal wall is indicated.

### Early Work on Humans

As early as 1876 Tietze (34) advised the use of omental grafts to reenforce end-to-end entero-anastomoses, for closing small perforations of the stomach, and for covering necrotic patches which he had experimentally produced on the surface of the intestines. But it remained to Chaput to make one of the early observations on the adhesiveness of omentum in humans in 1891 He had tried to repair losses of substance in the intestine with fragments of iodoformed gauze which was gradually eliminated by the intestine without accident. He observed that the omentum occluded the intestinal orifice, becoming grafted to the gauze and to the intestine (35)

Lanz (36) in 1892 made the first practical application of a free homoplastic hernial sac to cover leg ulcers and areas of the skin from which Thiersch grafts had been removed At first he thought that there was a metaplasia of the endo-

thelium into an epithelium but later findings showed that progressive necrosis of the hernial sac always occurred. In a few cases autografts were used showing the same results as homograft.

*Hume* (37) (1893) *reported a case of a fixed* ma in which openings were made in the stomach wall, and in the intestine opposite to the mesentery. A continuous suture was run around the *margin of the opening in the stomach attaching* the mucous and peritoneal coats. Senn's plates were secured and a series of Lembert stitches were placed between the gastric and intestinal wall around the outer margins of the plates and a large piece of omentum completely detached was wrapped around the junction of the two viscera and fixed in position. Before the omental piece was placed the surfaces of the graft and of the viscera which were to be intact were lightly scarified. Two months later at autopsy the outline of the omental graft could still be traced.

Bennet (38) (1890) covered a perforated peptic ulcer successfully with free grafts of omentum. In 1899 Berezanowsky employed a freshly excised hydrocele sac for interposition between the bony covering of the brain and the pia in order to *prevent adhesions after operation on the skull* (39).

In his experiments on the liver in animals Loewy (11) described procedures which he believed were indicated especially in deep lesions of the liver and spleen in humans. These methods of peritoneal application had not been carried out in a sufficiently large number of human subjects at the time 1901 to be included in this report. He recommended the application of a section of the peritoneum to the lesions of the visceral organs as a method of securing wound sutures, for example of the intestine and liver, and also as a method of establishing or completing hemostasis, of obliterating an opening or of rendering denuded surfaces fit for artificial stumps. Tuffier also (1903) reported successful use of free omental transplants in lesions of the liver and spleen (40).

Mauclaire did not venture to try cutting the pedicle as recommended by Loewy when em*ploying pedicled omentum for insertion into a* pocket in the peritoneal cavity where a hydatid intrahepatic cyst had been located. The cyst was complicated by cholerrhagia. The graft arrested the cholerrhagia and activated the filling of the cavity. Mauclaire further obtained an excellent result *from the use of omentum implanted in a* patient with bleeding from the lacerated liver (41). In 1904 he reported a case of homologous omental graft taken from an adolescent and transplanted into the tibia of a child with osteomyelitis. *The wound did not heal and the omen*tum was eliminated in the form of oily liquid. This appears to be the first attempt to graft homologous tissue into the human (42).

*Lebreton (1904) employed free omental trans*plants in lesions of the liver and spleen, with good results (43). When unable to close the perforation by sutures in a patient with duodenal ulcer Clogg (1905) brought up the free edge of the omentum and closed the lesion by suturing the attached omentum around the perforation, with excellent result (44). Bougle in 1906 covered a bleeding lesion of the spleen successfully with free omental flaps (45).

Peritoneum was used by Kocher in 1907 for the replacement of dural defects (46) and was recommended as a layer between the bony covering and the pia to prevent adhesions following operation on the skull. Lanz in the same year reported the use of hernial sac as suture material in hernia plasty (8). Seuckler (47) (1908) wrapped omentum *around portions of the intestine from* which the blood supply had been cut off.

The first comprehensive studies on the use of transplantation of peritoneum were carried out by von Hacker and his associates in 1908 and earlier. In numerous cases hernial sacs were employed as separation material in order to prevent postoperative adhesions after tendon and nerve repair or after freeing of adhesions between muscle and bone. They were also used to protect tendons and nerves freed from adhesion by forming a sheath to prevent new adhesions (48). In 1910 he used hernial sacs as transplants and considered them to be only a substitute for living tissue (49).

Lexer (50) (1909) was the first to attempt the clinical transplantation of a hydrocele sac as substitute for a joint capsule. This resulted in firm adhesions.

Boljarski (51) in 1910 reported successful control of bleeding by the use of free omental graft *in cases of laceration or resection of the liver.* A few years later he was able to report on 18 cases in which he covered the raw surface of the liver with grafted omentum (52). Hesse (1910-1911) also carried out successful repair of lacerated liver with free omental graft in ten in-

stances while in 79 patients he merely applied a tampon with a mortality of 20 per cent. Later he reported two instances of treatment of a lacerated spleen by this omental graft procedure. He treated ruptures of the liver in like manner (53)

Potherat (54) (1911) reported a favorable course in a case presented by Cantas of Athens in which an osseous cavity produced as a result of osteomyelitis of the tibia was filled with omentum resected from an epiplocele from another patient. The graft was not infected at the time of reporting and was not eliminated

After removal of a large tumor from the liver Clairmont (1911) controlled the bleeding by covering the defect with the peritoneal surface of the removed gallbladder with favorable result (55) Also in 1911 Deutschländer (56) used fresh homoplastic hernial sac after removal of ankylosis of the knee, with good results.

Kolaczik (22) in 1912 used hernial sac quite successfully as a tendon sheath in a patient and transplanted fresh homoplastic hernial sac for a defect in the periosteum of another patient with traumatic exostosis. He believed that the grafts were largely preserved as such after transplantation but conceded that considerable primary degeneration occurred. He described extensive adhesions following transplantation of peritoneum not only in the peritoneal cavity but also in joints. Histologically replacement by complete fibrous tissue ultimately supervened after grafting of peritoneum. He covered the suture line of nerve by a transplant of peritoneum and also used it for interposition between skin and bone in order to prevent postoperative adhesions of the skin.

Stuckey (57) (1912) controlled persistent bleeding after cholecystectomy by a free transplant of omentum pressed against the raw surface of the hepatic wound. At autopsy three days later the graft was observed to be adherent to the liver bed and there was no leak between the peritoneum and the liver tissue. He advocated free omental transplants because the material is easily accessible in any amount and because of the adhesive quality of this tissue In two patients the transplantation was successful and without complications In the same year Islin (58) covered a raw peritoneal surface upon the mesentery with a free omental palm-size graft, and found at autopsy seven days later that the transplant had not only grown in place but its surface was glistening and free from adhesions.

Judd (59) while performing a mastectomy for benign tumor of the breast operated on a large irreducible umbilical hernia. From the hernial sac he obtained a suitable mass of peritoneum and implanted it at the site of the mastectomy The graft remained viable and the cosmetic result was gratifying.

Fresh homoplastic hernial sac was used by Léonté (60) in 1914 as tendon sheath after loosening of tendons and nerves from scar tissue, with good functional results. Mühsam (1914) was able to control severe bleeding from a shot wound in the spleen by drawing a free omental strip through the shot canal and suturing the ends of the strip together (61)

Ohkohchi (62) in 1914 found that a free transplant of omentum applied to bleeding lacerated organs was much congested with blood after three days and adhered to the subtratum and finally was lifted by hematomas. The connective tissue of the organs grew out rapidly and produced hematoma, whereas growth of connective tissue of the graft was noted after 30 days. He believed that the hemostatic effect was mechanical and that secondary bleeding after free transplantation of omentum could not be avoided In his experiments Beresnegowsky (63) (1914) obtained results similar to those of Ohkohchi He formed a tampon of omentum and in addition sutured omentum over this tampon and still secondary bleeding occurred He therefore recommended the use of a free transplant of omentum together with deep mattress sutures.

In 1916 Binnie (64) recommended use of omental grafts to cover stumps of fatty mesentery reenforcing the suture with free omentum. He expressed preference for the use of free omental grafts to close the perforation of duodenal ulcer After resecting several feet of small intestine because of a growth and other lesions, he advised spreading a suitable portion of omentum over the raw stump fixing it by a few sutures and trimming. Where it is impossible to cover raw surfaces in instances of obstructive adhesions with neighboring peritoneum he thought that portions of free omentum may be plastered over the surface.

An omental graft was used by Freeman (65) in 1916 to cover a large raw surface resulting from the 'unfurling of a Lane's kink and to replace lost portions of the intestinal peritoneum

resulting from separation of adhesions. He preferred the use of a free peritoneal graft because an attached graft may give rise to entangling bands or to injurious traction upon the colon, the duodenum or the stomach.

Castro (66) of Costa Rica (1916) reported a case in which the patient had been operated for multiple uterine fibroma four years previously. At operation the omentum was found to be irregularly thickened and adherent to the abdominal wall and intestine. The appendix having been removed a very thick adhesion was encountered between the ileum and peritoneum over the right sacroiliac synchondrosis and another one between two loops of small intestine. To avoid formation of an obstruction after removal of Meckel's diverticulum a piece of mesentery between two veins was dissected to cover the stump and a denuded area of the bowel the raw surface of the transplant being in contact with the defect on the intestine. Recovery was uneventful.

Wederhake (67) in 1917 reported several successful cases in which he used hernial sac as tendon sheath. He operated successfully on two patients with Dupuytren's contraction by this method. He also employed hernial sac to cover defects of the periosteum and to fill cavities within bone. An osseous cyst healed after being filled with hernial sac. In the transplantation of hernial sac into wounds and granulating surface, Wederhake thought that the endothelium of the hernial sac took over the role of epithelium. In clean wound the results were always good. In some infected patients the hernial sac did not take but in hi opinion it is still of advantage as it serves a a stimulating irritant for the growth of epithelium. Wederhake used hernial sac in a variety of wounds after amputating a surface of the penis decubital ulcers defects of the mucosa of the lip of the cheek and nose and of the urethra.

Bier (68) in 1918 expressed the opinion that the endothelium in grafting hernial sac deteriorates rapidly. He observed no real takes only necrosis. In spite of thi the hernial sac serves a a suitable covering for wounds and ulcers since it act a a protective membrane. This according to Bier is the reason why ulcers heal more quickly under the covering of hernial sac.

Abdominal omentum was employed by Morris (69) in a patient with meningeal adhesion after injury to correct depression of a bon skin flap previously replaced. He state we employ grafts taken from other ind antibodies are called to the region "for pose of di posing of the stranger

Mann (70) (1921) advocated infinit the handling of omental transplant so t delicate cells would not be injured, an mended tucking in the edges so that the cut surface be exposed. In this wa possible to cover a traumatized area so i if any adhesions occurred

Writing in 1923 Neuhof (71) expr belief that an indication for clinical use transplantation of peritoneum has n established. The homograft in his ju undergoes degeneration, and the fibro ciated with it presents all the disadvant none of the compensating advantages grafts

Lexer (72) concluded from his clinical a his experimental observations that th plantation of peritoneum in all in t covering defects of the blood vessel tho skin and mucosa operative wound d the periosteum and as suture material in necrosis of the transplanted tissue. H fore did not recommend the use of per In other words he believed that free tran tion of any sort should not be used wi peritoneal cavity or outside of it

It is dangerous according to Lexer on an omental graft alone to control from parenchymatous organs. Secondar ing may rupture the capsule-like oment ing. Scars form and other parts of the o destroyed (72)

Graham (73) (1931) presented a clinical report of 20 cases of free omentu selected from the record at the M Hospital in Brooklyn, New York. Th were used in a variety of condition h the terminal ileum mesentery suture l liver and duodenum and including defe abdominal wall leaking ureter and defe gallbladder fossa, pylorus and duodenu ham concluded from the results that free grafts will live they are hemostatic the preserving peristal i they reenforce weal lines they resist infection. In three in a recorded the graft when examine found to be unimpaired

**Later Observations of Peritoneal Grafting in Animals**

Using dogs, Brackin (74) in 1940 included intact parietal peritoneum overlying the ureter in a lateral submucous ureterointestinal anastomosis. The ureterointestinal opening is established by an absorbable suture and the ureter above the necrosing suture is anchored in the intestinal incision, the sutures including, on the medial side of the ureter, the parietal peritoneum and the inferior edge of the intestinal muscle. The suture on the lateral side of the ureter includes the parietal peritoneum and the superior edge of the intestinal muscle. The intestinal muscles are closed over the ureter at the suture site, and folds of parietal peritoneum remain between the approximated edges of the intestinal wall. The field is peritonealized.

Films of specimens of kidneys, pelves, ureters and sections of the bowel taken from six dogs were within normal limits.

In ten bilateral implantations in healthy dogs no pathologic changes in the kidneys or in the ureters were observed after periods of from 3 weeks to 11 months. The results obtained were good.

In Brackin's opinion the incidence of hydronephrosis and pyelonephrosis following implantation of the ureters into the colon has been reduced experimentally by this method which involves utilization of peritoneum and in which dissection of or trauma to the ureter does not occur.

In 1942 McGehee and Tendler (75) subjected dogs to laparotomy and applied free omental grafts to scarified areas. Four out of five dogs showed perfect results. Of the dogs subjected to free omental grafts in which the peritoneum was not injured, all showed poor results.

Of nine dogs in which free transplants of peritoneum were employed by Devine (76) (1946) to cover anastomoses of sectioned colon, all grafts were found to be adherent at least in part.

Chester Bell and McCorkle (77) (1949) experimenting with dogs, removed a rectangular graft of peritoneum 1 by 3 inches (which included some or all of the thin fascia of the posterior rectus sheath) from the lateral aspect of the laparotomy incision. End-to-end anastomosis of a divided loop of colon was carried out. The free peritoneal graft was placed around the anastomosis, peritoneal side down, and sutured in position. The grafts remained completely viable and adherent in every animal, some from 44 to 120 days postoperatively. The removed specimens showed the grafted area still thickened but otherwise indistinguishable from the normal serosa and subserosa of the intestine; the sutures were surrounded by fibrous tissue. The outer raw surface was covered with epithelium as early as the twelfth postoperative day. No constriction occurred at the site of the intestinal anastomoses. The results were satisfactory, the experiments demonstrating the part a free graft alone plays in sealing off the site of anastomosis.

After simple division of the esophagus in dogs, end-to-end anastomosis in two layers was performed by Kleinsasser, Cramer and Warshaw (78) (1950). Peritoneal grafts taken from the abdomen on the operative side were applied to the line of anastomosis, with the serosa turned inward. There appeared to the authors to be no particular advantage in using grafts, as 2 of 7 surviving grafted animals developed leakage while only one control showed the same result. The grafts, however, became intimately adherent to the esophagus and were microscopically indistinguishable from the adventitia.

In experiments on dogs, Mighetta and Noli (79) (1951) resected the cervical esophagus and then brought the parts together in end-to-end anastomosis. Free peritoneum was drawn through the right parietal incision and sutured over the line of esophageal anastomosis. The suturing technique was varied in different groups of dogs. The authors believe that the use of free peritoneal transplant accelerates the healing of the line of anastomosis and strengthens the cicatrices. The graft of peritoneum, however, is unable to prevent dehiscence and fistula when the technique is not suitable.

Full thickness circular defects, 1 cm. or more, were made by Moore and Singleton (80) (1951) on the lateral surface of the stomach or intestine in dogs by excision of all layers. A peritoneal graft taken from the parietal peritoneum adjoining a rectus incision was applied over the defect and secured to the serosa of the viscus. In other dogs similar defects were made in the thoracic esophagus and were covered with free transplants of pleura. In still other dogs a Polya type subtotal gastric resection was carried out, leaving the duodenal end inverted and open except for a peritoneal graft sutured around the serosal cuff of the duodenum. In some survivors large open-

ings in the stomach, duodenum or esophagus *were sealed successfully with free serous grafts.* Microscopically the mucosa and muscularis had become approximated across the defect.

Verne and Perel (81) (1952) reported complete *fusion of free peritoneal graft to the intestinal* wall in dogs, and differentiation of certain of the graft elements into smooth muscular fibers. A section of the whole thickness of the intestinal *wall at the site of transplantation showed an* exact suture and a still recognizable section of the graft after a month. The animals did not succumb to peritonitis. Verne and Pérel believe *peritoneum to be not only an available serous* covering but a plastic material for reconstruction which is not negligible.

For the prevention of leakage Hinan (82) (1953) used a *free graft of posterior parietal* peritoneum, with or without fascia, taken from the wound to cover the site of direct uretero-intestinal anastomosis in dogs. Variations in method were: thin peritoneal graft without suture and with fixation, full thickness graft with fixation and a graft with plasma thrombin adhesive. Hinan concluded that a free graft of peritoneum at the site in experimental anastomosis was no better than a serosa-serosa re-enforcement (a second row of sutures) in clinical experience. The best method was the full thickness graft with fixation. When failure occurred, it resulted from abscess formation in the contaminated closed space beneath the flap.

Lodigiani and Esposti (83) (1953) carried out experiments on nephrectomized (on one side) dogs, removing a free piece of peritoneum, with fascia of the rectomuscle, and implanted it into a tract of the ureter on the opposite side with plasma-thrombin and sutured it. The endothelial surface of the peritoneal transplant was turned toward the canal of the ureter. Histologic examination from 10 to 15 days postoperatively showed the ureters to be normal and the peritoneal transplant to be absorbed. In a second group of dogs treated similarly but without application of plasma thrombin, at autopsy the ureter was found to be well patent and stretched and no peritoneal tissue was noted. The control animals died after formation of fistula.

In experiments on dogs, Hani and Pack (84) in 1956 reported on a study of the final results in peritoneal grafting on the raw *surface of the* liver. Through a right subcostal incision made in the abdomen a segment of peritoneum, ~ by 3 cm., was dissected from the left lateral abdominal wall. *The graft was then sutured to the superior* surface of the liver one centimeter from the margin of the left lateral lobe to be resected. After the liver was transected, the free border of the peri*toneal graft was laid horizontally across the raw* surface of the transected liver and sutured to the inferior surface 0.5 cm. from the edge. The animals were examined for a second time *at intervals of one to three months after the* liver resection and grafting. The peritoneal grafts in all animals were successful with no evidence of hemorrhage or peritonitis. In only *one of the dogs was there an adhesion between* the peritoneal graft and the intestine. Microscopically the peritoneal graft almost simulated the normal capsule of Glisson; a loose connective *tissue layer intervened between the peritoneal* covering and the underlying normal liver tissue.

Since large mucosal defects are quite common following surgery in the oral cavity, a recent attempt was made by Richard Brasfield (85) to find a suitable tissue for use in transplantation. The buccal mucosa of dogs was excised on both sides. Fresh free peritoneal grafts, 3 to 5 cm., were sutured over each denuded surface. All grafts survived. After one month microscopic sections were taken. The transplanted tissue could not be distinguished from normal buccal mucosa. After two months the grafts contracted approximately 20 per cent but it was difficult to identify the site of the graft.

## Later Observations of Peritoneal Grafting in Humans

McGehee and Tendler (75) (1952) used free omental grafts in 13 cases of adhesive obstruction and in areas of potential obstruction due to denudation of visceral or parietal peritoneum. In none of the cases reported did the clinical results require further abdominal surgery. Grafts were taken from the thinnest portion of the omentum. McGehee and Tendler buried the cut edges and vascular bundlings and removed no more omentum than was necessary. The 13 patients whom they treated with this technique remained clinically well after operation. They believe that free omental graft is "a rational homologous agent, easily available and easily utilized, in patients with adhesions, with adhesive *obstruction, or with other conditions in* which areas of denudation of peritoneum occur."

In Schmidt's opinion (86) vascular peritoneum

is suitable for covering areas in the small pelvis. It is generally sufficient to draw a fold of vesicular peritoneum over the suture line on the base of the uterus or on a stump of the uterus extending to the posterior wall of the uterus or into the fold of Douglas. But occasionally a large wound surface on the posterior wall of the neck of the uterus and in the fold of Douglas (e g after removal of spreading endometric foci in a younger woman) cannot be covered with healthy Fallopian tubes. Then vesicular peritoneum turned 90 degrees as a pedicled flap may be sutured in the peritoneal defect. In 12 women operated on by this method a good permanent result was obtained.

In 1946 Garat and Frias (87) reported the case of a patient who had two abdominal fistulas, a sigmoidal cutaneous one deeply adherent extending in the medial part of an infraumbilical operative scar and another fistula corresponding to a cecostomy These authors applied a flap of peritoneum to the colon and sutured an extraperitoneal closure of the sigmoidal orifice of the fistula to the border of the colon A few weeks later the cecal colostomy was closed The fistular canal showed a fibrous process. Six months postoperatively the patient continued in good condition and both fistulas were firmly closed. Radiographically the sigmoid colon was penetrable. Garat and Frias consider peritoneal plasty of surgical value in closing extraperitoneally a subcutaneous fistula in which the original orifice is separated from the anterior parietal plane or in which adhesions are present that will press the intestine deeply into the abdominal wall.

Devine (76) (1946) referred to a graft of whole thickness skin and a free graft of peritoneum being applied around an exteriorized loop of human colon on its peritoneal aspect. Plasma was applied to the bowel and a few drops of thrombin solution to the graft. The grafts were firmly fixed in 24 hours by the third day appeared to have a blood supply and by the fourth day the margins could not be lifted

Hogeman (88) (1948) reported two cases in which he used free transplant of omentum over defects of peritoneum from operative trauma to prevent postoperative adhesions. In one patient parts of the large bowel were adherent after surgery for salpingitis Serosal defects from loosening of the adhesions were closed with free transplants of omentum Recovery ensued without complications.

After appendectomy and removal of myoma from the uterus the second patient had an obstruction of the small intestine with adhesion to the cecal pole. A large peritoneal defect after loosening of adhesions was closed with a free transplant of omentum and sutured At autopsy following paralytic ileus the transplant was firm and sound Microscopically no inflammatory process was evident but there were small areas of granulated tissue.

Rosenstein (89) (1950) used the great omentum to cover peritoneal defects. He recommends transplantation of the great omentum in areas where the peritoneum is damaged

In the case reported by Rothman (90) (1951) obturator hernia was reduced in a 75-year-old woman and the appendix removed resulting in a defect in the obturator foramen A rectangular graft taken from the lateral aspect of the upper end of the laparotomy incision was sutured to the margins of the wound so that the peritoneal surface of the graft faced the peritoneal cavity The patient suffered no further difficulties.

A free fragment of omentum was introduced by Gaillard (91) (1952) into the opening of a perforated peptic ulcer in such a manner that 3 cm. of the omentum hung into the gastric cavity and the rest emerged from the opening The graft was fixed to the wall of the cavity

In 1953 Allen (92) ventured to use peritoneum in extensive full-thickness conjunctival defects to prepare for corneal transplants. In one patient, following enucleation of the eye because of injury a buried muscle-cone implant had been inserted. The socket contracted, associated with closed immobile lids and thick scar tissue After dissection of the scar tissue down to the tarsal plates, a molded dental wax stent was wrapped with peritoneal mucosa and inserted into the bed. After the eighth week the stent was removed and a conformer was given for wear Further removal of scar tissue was necessary before complete success was achieved In the second case a similar condition was satisfactorily repaired by the same method although the eye socket was immobile

Allen also includes two cases in which after superficial keratectomies and extensive removal of bulbar conjunctiva the defect was covered with peritoneal mucosa, followed by successful corneal transplantation

He points out the advantages of using peritoneal mucosa particularly omentum as a substitute for conjunctiva. It is moist and trans-

ings in the stomach, duodenum or esophagus were sealed successfully with free serous grafts. Microscopically the mucosa and muscularis had become approximated across the defect.

Verne and Perel (81) (1952) reported complete fusion of free peritoneal graft to the intestinal wall in dogs and differentiation of certain of the graft elements into smooth muscular fibers. A section of the whole thickness of the intestinal wall at the site of transplantation showed an exact suture and a still recognizable section of the graft after a month. The animals did not succumb to peritonitis. Verne and Pérel believe peritoneum to be not only an available serous covering but a plastic material for reconstruction which is not negligible.

For the prevention of leakage Hinan (82) (1953) used a free graft of posterior parietal peritoneum with or without fascia, taken from the wound to cover the site of direct uretero-intestinal anastomosis in dogs. Variations in method were thin peritoneal graft without suture and with fixation, full thickness graft with fixation, and a graft with plasma thrombin adhesive. Hinan concluded that a free graft of peritoneum at the site in experimental anastomosis was no better than a serosa-serosa re-enforcement (a second row of sutures) in clinical experience. The best method was the full thickness graft with fixation. When failure occurred it resulted from abscess formation in the contaminated closed space beneath the flap.

Lodigiani and Laposti (83) (1955) carried out experiments on nephrectomized (on one side) dogs, removing a free piece of peritoneum, with fascia of the rectomuscle, and implanted it into a tract of the ureter on the opposite side with plasma thrombin and sutured it. The endothelial surface of the peritoneal transplant was turned toward the canal of the ureter. Histologic examination from 10 to 15 days postoperatively showed the ureters to be normal and the peritoneal transplant to be absorbed. In a second group of dogs treated similarly but without application of plasma-thrombin, at autopsy the ureter was found to be well patent and stretched and no peritoneal tissue was noted. The control animals died after formation of fistula.

In experiments on dogs Islami and Pack (84) in 1950 reported on a study of the final results in peritoneal grafting on the raw surface of the liver. Through a right subcostal incision made in the abdomen a segment of peritoneum, 7 by 4 cm., was dissected from the left lateral abdominal wall. The graft was then sutured to the superior surface of the liver one centimeter from the margin of the left lateral lobe to be resected. After the liver was transected, the free border of the peritoneal graft was laid horizontally across the raw surface of the transected liver and sutured to the inferior surface 0.5 cm. from the edge. The animals were examined for a second time at intervals of one to three months after the liver resection and grafting. The peritoneal grafts in all animals were successful with no evidence of hemorrhage or peritonitis. In only one of the dogs was there an adhesion between the peritoneal graft and the intestine. Microscopically the peritoneal graft almost simulated the normal capsule of Glisson, a loose connective tissue layer intervened between the peritoneal covering and the underlying normal liver tissue.

Since large mucosal defects are quite common following surgery in the oral cavity, a recent attempt was made by Richard Brasfield (85) to find a suitable tissue for use in transplantation. The buccal mucosa of dogs was excised on both sides. Fresh free peritoneal grafts, 3 to 5 cm., were sutured over each denuded surface. All grafts survived. After one month microscopic sections were taken. The transplanted tissue could not be distinguished from normal buccal mucosa. After two months the grafts contracted approximately 20 per cent but it was difficult to identify the site of the graft.

## Later Observations of Peritoneal Grafting in Humans

McGehee and Tendler (75) (1943) used free omental grafts in 13 cases of adhesive obstruction and in areas of potential obstruction due to denudation of visceral or parietal peritoneum. In none of the cases reported did the clinical results require further abdominal surgery. Grafts were taken from the thinnest portion of the omentum. McGehee and Tendler burned the cut edges and vascular bunchings and removed no more omentum than was necessary. The 13 patients whom they treated with this technique remained clinically well after operation. They believe that free omental graft is "a rational homologous agent," easily available and easily utilized in patients with adhesions, with adhesive obstruction, or with other conditions in which areas of denudation of peritoneum occur.

In Schmid's opinion (86) vesicular peritoneum

is suitable for covering areas in the small pelvis. It is generally sufficient to draw a fold of vesicu lar peritoneum over the suture line on the base of the uterus or on a stump of the uterus extending to the posterior wall of the uterus or into the fold of Douglas. But occasionally a large wound surface on the posterior wall of the neck of the uterus and in the fold of Douglas (e.g., after removal of spreading endometric foci in a younger woman) cannot be covered with healthy Fallopian tubes. Then vesicular peritoneum ...med 00 degrees as a pedicled flap may be ...tured in the peritoneal defect. In 12 women ...perated on by this method a good permanent ...esult was obtained.

In 1916 Garat and Frias (87) reported the case of a patient who had two abdominal fistulas, a sigmoidal cutaneous one deeply adherent extending in the medial part of an infraumbilical opera tive scar and another fistula corresponding to a cecostomy These authors applied a flap of peritoneum to the colon and sutured an extraperitoneal closure of the sigmoidal orifice of the fistula to the border of the colon. A few weeks later the cecal colostomy was closed The fistular canal showed a fibrous process. Six months postoperatively the patient continued in good condition and both fistulas were firmly closed Radiographically the sigmoid colon was penetrable. Garat and Frias consider peritoneal plasty of surgical value in closing extraperitoneally a subcutaneous fistula in which the original orifice is separated from the anterior parietal plane or in which adhesions are present that will press the intestine deeply into the abdominal wall.

Devine (76) (1946) referred to a graft of whole thickness skin and a free graft of peritoneum being applied around an exteriorized loop of human colon on its peritoneal aspect. Plasma was applied to the bowel and a few drops of thrombin solution to the graft. The grafts were firmly fixed in 24 hours, by the third day appeared to have a blood supply and by the fourth day the margins could not be lifted

Hogeman (88) (1948) reported two cases in which he used free transplant of omentum over defects of peritoneum from operative trauma to prevent postoperative adhesions. In one patient parts of the large bowel were adherent after surgery for salpingitis Serosal defects from loosening of the adhesions were closed with free transplants of omentum Recovery ensued without complications.

After appendectomy and removal of myoma from the uterus the second patient had an obstruction of the small intestine with adhesion to the cecal pole A large peritoneal defect after loosening of adhesions was closed with a free transplant of omentum and sutured At autopsy following paralytic ileus the transplant was firm and sound Microscopically no inflammatory process was evident but there were small areas of granulated tissue.

Rosenstein (80) (1950) used the great omentum to cover peritoneal defects. He recommends transplantation of the great omentum in areas where the peritoneum is damaged

In the case reported by Rothman (90) (1951) obturator hernia was reduced in a 75-year-old woman and the appendix removed, resulting in a defect in the obturator foramen. A rectangular graft taken from the lateral aspect of the upper end of the laparotomy incision was sutured to the margins of the wound so that the peritoneal surface of the graft faced the peritoneal cavity The patient suffered no further difficulties.

A free fragment of omentum was introduced by Gaillard (91) (1952) into the opening of a perforated peptic ulcer in such a manner that 3 cm. of the omentum hung into the gastric cavity and the rest emerged from the opening The graft was fixed to the wall of the cavity

In 1953 Allen (92) ventured to use peritoneum in extensive full thickness conjunctival defects to prepare for corneal transplants. In one patient, following enucleation of the eye because of injury a buried muscle-cone implant had been inserted The socket contracted associated with closed immobile lids and thick scar tissue. After dissection of the scar tissue down to the tarsal plates a molded dental wax stent was wrapped with peritoneal mucosa and inserted into the bed. After the eighth week the stent was removed and a conformer was given for wear Further removal of scar tissue was necessary before complete success was achieved. In the second case a similar condition was satisfactorily repaired by the same method although the eye socket was immobile

Allen also includes two cases in which after superficial keratectomies and extensive removal of bulbar conjunctiva the defect was covered with peritoneal mucosa followed by successful corneal transplantation.

He points out the advantages of using peritoneal mucosa, particularly omentum as a substitute for conjunctiva. It is moist and tra

parent is relatively easily separated from its submucosal tissue and is available in sufficient quantity to replace the entire conjunctiva of one or both eyes, if necessary

In 1956 Kamienski (93) repaired fresh and granulating skin losses with homoplastic peritoneal transplants and fresh or preserved grafts of hernial sac In 33 cases he transplanted peritoneum on the granular skin of denuded areas or on fresh wounds which could be covered only by a plastic procedure He used peritoneum obtained from herniorrhaphy for hydrocele of the testicle and funiculus spermaticus in adults or children. The peritoneum was transplanted several minutes after it had been obtained or after preservation in physiologic salt solution plus penicillin for 20 to 30 hours. The flaps of peritoneum were applied on the granular surface where skin loss occurred or its serous surface or the surface of the connective membranes. In some patients Kamienski sutured the margin of peritoneum to the skin margins with knot sutures. He observed that the flaps of peritoneum did not heal by the same process as that of a free transplant of autogenous skin. The surface of the flap underwent dry necrosis and a dry scab formed. No particular differences in the behavior of the surface of the flaps were seen whether the flap was applied with the external or internal surface of the peritoneum to the wound The fresh flaps of peritoneum transplanted immediately after being obtained healed better than the transplants which were preserved by refrigeration.

Kamienski draws the following conclusions The transplant of peritoneum may be used to cover fresh granular surface after skin loss and also to cover a slowly healing ulcer because it markedly accelerates growth of the epidermis and favors healing of the wound. The transplanted peritoneum fuses actively with the granular base and represents the source of the membranous transplant which later grows out and quickly covers the skin loss The transplanted flap of peritoneum does not grow entirely to the base and is not a substitute for the skin The blood vessels grow into the transplant rapidly which for the most part undergoes resorption. The tran planted peritoneum protects the wound from external influences and prevents wa te of fluid by the organism The result of applying a free flap of peritoneum taken from the hernial sac is favorable in small children, for whom it is difficult to obtain skin for coverage in cutaneous loss.

Islami and Pack (84) (1956) report that they have successfully used free omental grafts and free parietal peritoneal grafts on liver wounds. They believe that a large segment of the abdominal wall may be safely denuded of its parietal lining for grafting because it regenerates rapidly and often with few and uncomplicating adhesions

## CLINICAL USE OF PERITONEAL TRANSPLANTATION

*Free transplantation originated from pedicle* transplantation Hence the action and results in free transplantation are identical with those in pedicle transplantation. Since pedicle transplantations can give rise to adhesions and obstruction phenomena, including strangulation of *the intestine they are used only where there is* no danger of such occurrences. The pedicled transplant has another limitation distance. Thus free transplantation, used experimentally by Chaput (1872) and then clinically by Senn (1892) was first applied to the problems of gastrointestinal surgery In his experimental work Loewy in 1901 employed free omentum to staunch bleeding from parenchymatous organs and Mauclaire applied it in clinical practice for the same purpose. With the development of the methods of free transplantation, the possibilities of the peritoneal graft increased it was used in defects of the brain and dura in joint capsules and skin defects. However Lexer and Dault later considered the dural use of peritoneum unwise they were also apprehensive about the procedure of omental ligation because of such occasional complications as thrombosis embolism and hemorrhage. Kehr (94) rejected free transplantation of omentum and called it *ultimum refugium* Freeman did not con ider the procedure dangerous and he tried to avoid necrosis by not ligating the larger vessels of the omentum. He recommended the use of tissue from the free edge of the omentum a suggestion which was widely adopted in clinical practice

### *Types of Free Transplants*

There are various types of transplants—autoplastic homoplastic or alloplastic and heteroplastic—according to the source of the tissue Some surgeons have used preserved dead homoplastic transplants and have tried to prove that since such material is not dependent on

nourishment from the surrounding tissue, it causes no adhesions. Autoplastic peritoneum however has proved to be the best tissue for transplantation on the abdominal organs. In our recent experiments we exclusively worked with autoplastic transplants and observed the behavior of transplanted peritoneum under the most favorable conditions.

**Free Peritoneal Transplants in Gastrointestinal and Esophageal Surgery**

Since the early days of modern surgery the protective role of peritoneum has been recognized as, for example, in the use of omentum to prevent the spread of gastrointestinal contents in perforation of the gastrointestinal tract. As long ago as 1002 Nicholas Senn (12) urged the use of omental transplantation for additional security in managing wounds or suture lines in the stomach and intestine Sealing of a large opening in the stomach, duodenum or esophagus with free serous grafts has occasionally been recommended for reenforcement purposes For example, Moore and Singleton (80) have used a free graft of peritoneum sutured in place over the line of intestinal inversion to reenforce the closure. It is a simple matter after closure of the duodenal stump in gastrectomy to excise a circular or rectangular patch of parietal peritoneum and tack it in place with a few sutures. Moore and Singleton have shown that in some instances when the duodenal stump has been reenforced by the use of a free peritoneal graft there has been no clinical evidence of subsequent leakage from the suture line. They point out that "the duodenal stump had first been inverted with traditional sutures and probably would not have leaked without the reenforcement. However when the surgeon lacks confidence in the security of an intestinal suture line, the use of a free graft of peritoneum is advisable."

It may be said that peritoneal transplants are applicable to all gastrointestinal anastomoses including esophageal anastomosis, gastroenterostomy closure of the duodenal stump and small bowel anastomosis.

As expressed by Devine (76) the problem of suturing the large intestine may be compared to repairing the inner tube of a motor tire where sutures cannot be employed effectively to repair rents because each stitch put into the rubber tube would produce leakage of air Therefore patches are cemented over the holes Analogously

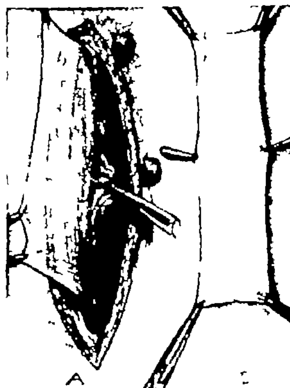



Fig 215 A Removal of peritoneal graft from lateral edge of laparotomy wound B Peritoneal graft after removal from abdominal wall From Spencer T Chester H Glenn Bell and H J McCorkle Surg Gynec & Obst 89 605 1949

the method of transplanting peritoneum over gastrointestinal anastomosis involves cementing' a peritoneal graft to the suture line whether or not the surface is covered by peritoneum. This transplantation of peritoneum on the anastomosing surface is a procedure supplemental to the usual method of anastomosis. It takes very little additional time at operation and there is nothing to lose if it fails.

In gastrointestinal surgery each suture hole may become infected and therefore may be a potential source of sepsis or leakage. Thus any sutured anastomosis of the gastrointestinal tract involves an element of risk. When peritoneum is sutured to peritoneum leakage is *ipso facto* minimized. However while a free transplant of omentum may be of value in reenforcing the suture line of a gastrointestinal anastomosis it does not eliminate the necessity for careful use of a regular technique in suturing

Saint and Mann (95) in 1929 listed the problems which make esophageal surgery more difficult than gastrointestinal surgery 1) the anatomic location 2) lack of a true serosa 3) poor blood supply 4) movement of the esophagus on respiration and deglutition, and 5) inability




Fig 216 A Site of division of colon B One layer closed end to-end anastomosis interrupted 0000 cotton sutures were used From Spencer T Chester H Glenn Bell and H J McCorkle Surg Gynec & Obst 89 003 1949

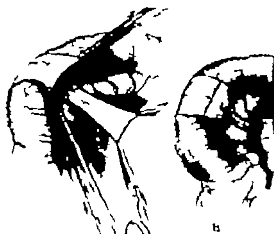



Fig 21 A Placing of graft around the site of anastomosis B Free peritoneal grafts sutured in position around site of anastomosis From Spencer T Chester H Glenn Bell and H J McCorkle Surg Gynec & Obst 89: 003 1949

of the omentum to protect and favor healing of the suture line When the intestine is sutured, its serosa seal the line of anastomosis by pouring out lymph over the area of the suture line Saint and Mann emphasized three important points in esophageal anastomosis (a) the use of interrupted sutures to conserve the blood supply of the anastomosis (b) sectioning of the left phrenic nerve to help in immobilizing the site of operation, and (c) prevention of alimentation and maintenance of nutrition intravenously keeping the esophagus at physiologic rest

There is no doubt that technical factors and *the skill with which the* anastomosis of an esophagus is done are essential to the success of the procedure Careful preservation of the blood supply and gentle handling are also very important. *Experimental and clinical studies* have shown that a single row of sutures is less likely to interfere with the blood supply of an anastomosis in the esophagus than multiple rows of sutures. It is conceivable that a double row of sutures may interfere with the blood supply to small areas about the anastomosis which may then necrose and leak. Consequently a single row of sutures with application of a peritoneal graft may be advisable.

As described by Devine (70) in the use of the free peritoneal transplant in gastrointestinal anastomosis the suture line may be very rapidly reenforced by a strip, about ³⁄₄ inch wide of *parietal peritoneum sufficient in size to pass* around the bowel with a little to spare The peritoneal graft should be moistened with a few drops of a solution of 5000 units U.S P of thrombin in 5 cc of saline The suture of the anastomosis should be swabbed with pooled human plasma which is readily obtained from a blood bank. *Then the graft is applied to the anastomotic line* Both the solutions may be kept for a long period of time in refrigeration even indefinitely

Usually if the free transplant of peritoneum is *applied on the anastomotic area* with pressure for 4 or 5 minutes the graft adheres readily and becomes firmly fixed in 24 hours *If handled roughly the graft may be rubbed off before becoming attached* After transplantation on the bowel for 48 hours the graft appears to be edematous and remains so for a few days By the third day the graft appears to have a blood supply and by the fourth day the edge cannot be lifted

### *Use of Omental Transplant in Preventing Adhesions*

The problem of checking or limiting intestinal adhesions following abdominal surgery is of such great practical importance that it has stimulated *many surgeons to find a solution.* The process underlying the formation of adhesions is a part of the normal repair of all wound of serous surfaces and consist of an outpouring of the plastic lymph which seals the lips of the wound. The problem therefore is not the prevention of

adhesions but the limitation of adhesions to physiologic requirements.

This problem of limiting adhesions therefore becomes a somewhat delicate problem of permitting the necessary adhesions and preventing the unnecessary ones. It does not seem that this problem can be solved by the use of any chemical or physical method It may be said that all wounds of the peritoneum must heal by a process of lymph formation which when carried too far results in adhesions. The only method of limiting adhesions is to limit wounds of the peritoneum, and this can be done by careful technique and by covering the necessary wounds with a free or attached portion of omentum or peritoneum. For limiting adhesions the surgeon should understand clearly that the peritoneum is not a structure which may be freely cut and sewn but a single layer of delicate endothelial cells that the cytologist obtains these cells for study by gently wiping the peritoneal surface with gauze sponge then pressing this sponge on a cover glass and that every wound of this layer of cells begins to heal by the fundamental process of adhesion formation—the outpouring of a plastic lymph (26)

A free graft of omentum has been used by various investigators under many experimental conditions in order to prevent adhesions in the peritoneal cavity With careful handling of the transplant so that the delicate cells will not be injured, and by tucking in the edges so that none of the raw surface is exposed, it is possible to cover the traumatized area so that few if any adhesions will occur A free transplant of omentum may be used over an area from which the peritoneum has been removed, and may be carefully sutured in position, no cut edges being left exposed in the peritoneal cavity This replaces the lost peritoneum to a certain extent. It should be emphasized, however that unless great care is exercised in transplanting the omentum, the adjacent organ will become adherent to the site of transplantation over a larger area than if the transplant had not been used. By exercising great care it is possible to use a free omental transplant to prevent adhesion but the value of such a procedure is greatly decreased by the fact that with careless handling the results, in all probability will be worse than if the transplant had not been used.

A free transplant of omentum may be employed partially to replace lost peritoneum but it is of no value and, again unless great care is taken the result may be worse than if the area had been left denuded

After *myomectomy* the sutured uterine wound may be covered with a plaster consisting of a free flap of omentum or peritoneum held in place by a few sutures. This graft aids in preventing any oozing of blood and also may prevent postoperative adhesions. When this operation is done to remove a large fibroma from the fundus of the uterus, it is sometimes impossible to suture the peritoneal surface of the uterus because of continuous bleeding hysterectomy in this instance may be the best procedure for producing hemostasis. If the patient is a very young woman however peritoneal grafting may be done and the organ saved.

*Histologic Observations*

In the first hours following peritoneal grafting on the stomach autopsy of experimental animals shows that the peritoneum is already becoming adherent. There is a thickening of the wall of the stomach in the region of the anastomosis. In the first few days the omentum has a reddish brown color and there is cicatrization of the mucosa which becomes continuous, starting from the fourth day Considerable congestion of the muscularis mucosae is evident. Thickening of the omentum occurs which is followed by atrophy or diminishing of the thickening and at about three and one-half months complete healing can be observed. From the sixth day the signs of cicatrization become manifest the submucosa is thickened and the fibrous tissue increases the muscularis mucosae is thin and has almost completely disappeared at this time. Large numbers of giant cells can be seen under the mucosa, as well as mononuclear cells in between the layers of the fibrous tissue.

There is nothing particularly noteworthy about the changes in the omentum. After one month there is a large amount of homosiderin pigment and a gradual increase of fibrous tissue. A small amount of fatty cells still persists. Formation of giant cells is observed around the suture material. At later periods the omentum completely disappears.

The interesting points to be noted in the process of cicatrization are the rapidity of healing of the mucosa, the formation of giant cells around the foreign bodies included in the different layers of the organ and the slight leuko-

denuded liver areas in dogs. In 15 dogs they applied the split thickness grafts to the transected middle lobe of the liver. On reexploration two weeks later the grafts were all found to have been successful. One to four months later the dogs were sacrificed and the skin grafts on the liver examined. All grafts remained intact but had markedly contracted to 30 per cent of their original size. Small cysts containing a squamous cellular detritus were found where the grafts overlapped the parietal peritoneum or liver capsule.

In human subjects the present author has never employed split thickness skin grafts on the liver but has successfully used free omental grafts and free peritoneal grafts from the abdominal parietes. A large segment of the abdominal wall may be safely denuded of its peritoneal lining because it regenerates rapidly and often with few and uncomplicating adhesion. The employment of such free peritoneal grafts as hemostatic coverings for liver wounds follows not only major lobectomies but also metastasectomies which are being done with increasing frequency. It is believed to lessen the quantity of postoperative bile drainage from the liver wound.

The various steps in free peritoneal grafting on a raw surface of the liver are diagrammatically demonstrated in figure 220.

The photograph of a free peritoneal graft (fig. 221) two months after it was applied to the transected left lateral lobe of the liver shows no evidence of liver contraction. No adhesions were observed.

*In the first hours following transplantation the* omentum simply becomes attached to the liver. The adherence to the liver is made possible either directly or intermediately by the fibrinous leukocytic exudate. By the middle of the second week there is a rich vascularization at the site of the grafting. The omentum becomes fibrous giving the impression of a fibrous capsule beneath the surface of the liver.

### *Histologic Observations*

Histologic sections from an omental graft on the liver in the first six hours show no modification in cicatrization. The blood vessels are still filled with blood and most of the fatty cells are absolutely normal. The fat does not undergo resorption. There is no sign of any fibrinous exudate between the liver and the graft. After the first few hours stealthy changes begin in the omentum and from the fourth day a thickening of the omentum is clearly visible. The nucleus of the fatty cells stains as brilliantly as previously.

A section of the liver of an animal sacrificed and examined four days after peritoneal grafting is shown in figure 222. The omentum shows two aspects: in one region the most remote from the liver it is essentially normal. There are however areas of necrosis around the suture line. In the region nearest the liver moderate thickening of the omentum can be seen. The vessels are greatly distended and there is a leukocytic infiltration. This leukocytic infiltration is more pronounced nearer the liver portion of the section; in certain areas however it is lacking.

There is some necrosis in the hepatic parenchyma in the sections taken from the suture line. *There appear to be two dominant factors:* 1) the existence of newly formed vessel and 2) leukocytic infiltration produced undoubtedly under the influence of germs or bacteria. The lower section (fig. 222) shows omentum with diminished fat cells in different areas. Transverse fibrous thickening of the omentum is already apparent. In the central part of the section marked thickening of a portion of the omentum is seen with leukocytic infiltration. In the right part of the section the omentum is directly adherent to the hepatic cells. The hepatic capillaries adjacent to the omentum are dilated and filled with red blood cells. Near the center *of the section there is a horizontal portion of* suture material above which marked capillary dilation is evident.

*In a histological section taken one month* after plastic operation (fig. 223) hepatic trabeculae are clearly visible in the upper portion. The remaining portion of the section is formed chiefly of omentum which has become fibrous. Here many vessels are seen, some filled with blood, others empty. Many giant cells are present. In the lower section of the omentum a fibrous thickening of the omentum is seen as pale undulations poor in cellular element, the interstitial tissue being infiltrated especially with leukocytes. At the junction of the liver and the omentum lies a very thick layer of conjunctive tissue poor in cellular elements communicating directly on the one hand with a fibrous conjunctive layer of the omentum, and on the other forming a fibrous capsular layer all around the hepatic cells.

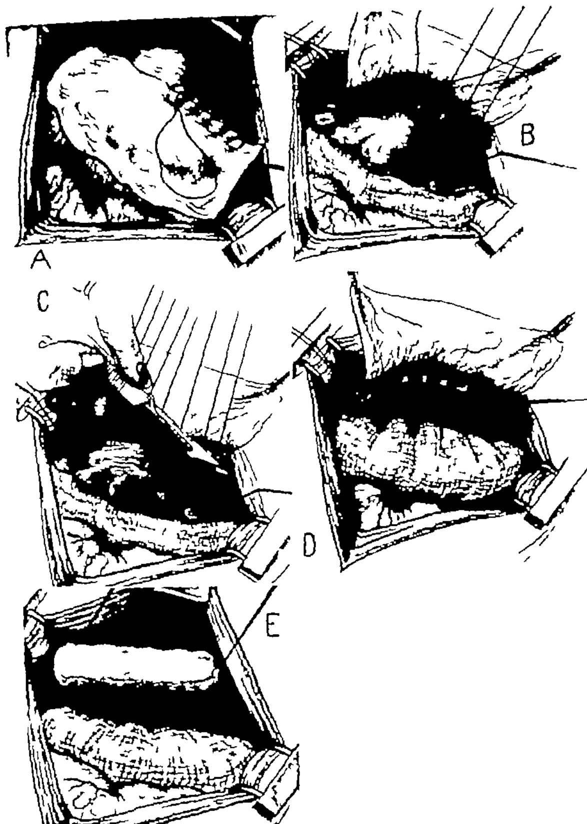



Fig 220 A Free peritoneal graft sutured to superior surface of liver First step B Free peritoneal graft sutured to superior surface of liver Preliminary mattress sutures inserted The segment of liver lobule becomes darker as the sutures are tied C Resection of the liver segment after the mattress sutures have been applied D The left lateral lobe of the liver has been transected. The peritoneal graft previously attached on the superior side is now ready to be applied over the cut liver surface E. Free peritoneal graft completely sutured over base of transected left lateral lobe



Fig 221 Two months after free peritoneal graft was applied to transected left lateral lobe of liver Note freedom of contracture and absence of *adhesions*



*Fig 222 Section of liver and omentum four* days after grafting From Robert Loewy Méthode des greffes péritonnéales Paris Thèse 1001



Fig 223 Section of liver and omentum one month after grafting From Robert Loewy Méthode des greffes péritonnéales Paris Thèse 1001



Fig 224 Liver surface at site of partial hepatectomy covered by a successful free peritoneal transplant two months before Note the loose *connective tissue of the peritoneum overlying the* hepatic parenchyma X275

### Attached Peritoneal Transplants

There is a wide range of possibilities for the use of attached omental graft in the peritoneal cavity provided care is exercised that such use does not furnish a basis for future intestinal obstruction. An attached transplant of omentum may be used to prevent adhesions to patch an opening *in some part of the gastrointestinal tract,* to strengthen a suture line in gastrointestinal surgery to replace lost peritoneum, to occlude a portion of the *gastrointestinal tract* and to *stop hemorrhage of a parenchymatous organ.*

Many surgeons use attached omentum to cover the repair of ruptured peptic ulcer the damaged area following cholecystectomy and choledochostomy over intestinal anastomosis a duodenal stump denuded parietal or visceral peritoneum around enterostomy tubes, and over abdominal incisions.

Pirone, Wilkie and Bost (97) recommend and have used attached omentum as a wrapping about a strangulated portion of the intestine the viability of which is doubtful or when the patient's condition does not warrant resection.

*Attached Omental Transplant to Prevent Adhesion* Saint and Mann (95) transplanted attached peritoneum into the peritoneal cavity to prevent adhesions. When the edge of the omentum, not in any way detached from its blood supply is sutured over any traumatized area in the peritoneal cavity the omentum adheres firmly to the traumatized area and thus prevents any of the adjacent organs from adhering to this area.

*Attached Omental Transplant to Patch Gastrointestinal Opening* It has been proved by Saint and Mann quite conclusively that attached omentum sutured as a patch over an opening in some part of the gastrointestinal tract has been of great value in preventing leakage from the lumen of the intestine into the peritoneal cavity

*Attached Omental Transplant to Replace Lost Peritoneum.* Undetached omentum may be sutured over a denuded area of peritoneum to prevent adhesions of other organs to the denuded area.

*Attached Omental Transplant to Control Hemorrhage* Attached omentum packed into a traumatized area of the liver kidney or spleen can produce complete hemostasis. Mann's experience indicates that great benefit is derived from the use of peritoneum in the control of hemorrhage from a parenchymatous organ. Byford (98) and Clark (99) however are reluctant to employ attached omentum to check bleeding because its attachment to the denuded or inflamed area may interfere with the normal physiology of the stomach and transverse colon. This may be true especially of its use in pelvic denudations or infection.

## Peritoneal Transplants Outside the Abdominal Cavity

If peritoneum is transplanted to a site outside the peritoneal cavity it no longer enjoys the favorable medium of peritoneal fluid which fills the cavity It is the lack of peritoneal fluid which makes a specific nourishment of the transplanted peritoneum impossible within the first hours after transplantation, that is, until the grafted peritoneum comes in contact with the interstitial fluid and the capillary system of its new environment. This lack of specific nourishing medium within the first few hours after transplantation tends to damage a rather tender tissue like peritoneum.

*Serous Cavities* Transplantation of peritoneum to other serous cavities is not feasible because the conditions prevailing within those cavities are not similar to those of the peritoneal cavity Regarding the use of peritoneal transplants on defects of the pericardium and of the parietal pleura, both experimental and clinical experience are evidently lacking.

*Ureters* After implantation of the ureters into the colon, as pointed out by Brackin (74) serious sequelae such as hydronephrosis and pyelonephrosis are possible. Interference with the function of the ureters is significant, for at least six factors are interrelated namely peritonitis, infection of the ureter and of the kidney impairment of function of the ureter retention of the urine and stricture and angulation of the ureter Brackin suggests a procedure in which intact parietal peritoneum overlying the ureter is included in the lateral ureterointestinal anastomosis.

The ureterointestinal opening is established by inserting a suture (no 6 braided silk material) through the parietal peritoneum and the ureteral wall on one side and the intestinal submucosa and mucosa on the other running parallel to the long axis of the ureter The desired length of the suture should lie in the lumen of the ureter This is accurately determined and the suture is tied securely with a crushing effect on the tissue. Care in manipulation is important to avoid breaking the suture material, injury to the parietal peritoneum and contamination, especially of the abdominal wound. The longitudinal ureteral vessels are avoided. The ureter above the necrosing suture is anchored in the intestinal incision by silk mattress sutures, which include, on the medial side of the ureter the parietal peritoneum and the inferior edge of the intestinal muscle. The suture on the lateral side of the ureter includes the parietal peritoneum and the superior edge of the intestinal muscle. Sutures

are not placed under the ureter proximal to the *side of the necrosing suture. The intestinal* muscles are closed over the ureter on the side of the necrosing suture and distally by silk mattress sutures, which include both edges of the intestinal muscle and pass under the ureter. *Folds of parietal peritoneum remain between the* approximated edges of the intestinal wall. The field is peritonealized. The right ureterointestinal anastomosis is performed first and the left in two or three weeks.

The peritoneal reaction prevents a *leakage* and tends to localize and absorb postoperative infection. The physiologic character of the ureter is preserved by deferring ureteral section and by implantation of the ureter with the nerves, blood vessels and parietal peritoneum intact. Stricture in the proximal segment is unlikely since the ureter is anchored in the intestinal incision by sutures in the parietal peritoneum. The probability of traction of the ureter is minimized by adhesions between the peritoneum and the intestinal submucosa. Obstruction of the small intestine after this procedure appears unlikely.

*Herniorrhaphy.* As has been previously stated, peritoneum has sufficient tensile strength so that it may be utilized in repair of certain inguinal and obturator hernias (90). Levering (33) showed that peritoneum will unite with fascia and when it comes in contact with the perimysium of muscle union occurs in a manner similar to that of union with fascial suture. So it is suggested that the hernial sac, which now is discarded, be used in operations where the reenforcement *of the abdominal wall is indicated.*

*Joints.* When a bony ankylosis has been divided and the articular ends of the affected bones have been properly modeled, union may be prevented and a new joint may be obtained by interposing *living tissue, such as peritoneum, between them.*

Lexer (5) in 1909 was the first to try the clinical transplantation of a hydrocele sac (homologous) as a substitute for a joint capsule. This attempt resulted in firm adhesions. Deutschländer *(8) in 1911 used fresh homoplastic hernial sac* with omentum after releasing ankylosis of the knee with good results and recommended the method. As reported by Kolaczek (22), two cases, involving the elbow joint and the hip joint *respectively, showed satisfactory result. In his* successful experiments on the knee joints in animals the transplanted tissue could not be grossly distinguished from the synovia. He found *that in the joint the peritoneum serves only as a* skeleton for the newly formed capsule. After two weeks the transplanted peritoneum was not present on the surface of the joint but had been pushed into deeper layers. The endothelium deteriorated soon and was replaced by the synovia.

In all the experiments and clinical cases of the author, firm adhesions resulted between the joint surfaces and the capsule associated with great loss of mobility. We cannot therefore recommend the use of free transplantation of peritoneum in the surgery of joints.

*Tendons and Nerves.* After tenorrhaphy or neurorrhaphy it is very important to prevent the line of union from becoming closely adherent to neighboring structures. To avoid this adherence wrapping a *free* flap of peritoneum around the united part as a prophylactic measure may be advisable.

*Conjunctiva.* Allen (92) considers peritoneum to be a satisfactory and adequate source of mucous membrane for use in extensive conjunctival defect. Peritoneal mucosa, particularly omentum, has several desirable characteristics for this purpose. First, it is moist, transparent, separable with relative ease from its submucosal tissue and available in sufficient quantities to replace the entire conjunctiva of one or both eyes. The elective removal of a piece of omentum from the normal abdomen does not appreciably increase the surgical risk of an extensive reconstruction of a large conjunctival defect, neither should it prolong the convalescence. Peritoneum may therefore be used as a substitute for conjunctiva.

As pointed out by Allen, the peritoneum may be used in cases in which the other procedures do not provide an adequate amount of mucous *membrane. The success of the operation depends* upon the immediate availability of an amount sufficient for replacement of a large amount of conjunctiva. With aseptic technique and the use of antibiotics the risk of infection of peritoneum *is negligible.*

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Kamieński (93) has presented the difficulties of covering losses of skin in small children. He points out certain known facts. Covering a large area of loss of skin with a transplant is beneficial to the course of recovery and reduces the time required. Only autoplastic transplants are used successfully and to take such a graft from the skin is not only difficult but harmful to a child. The value of fresh or preserved homoplastic skin is limited, except in monozygotic twins, while heteroplastic transplants do not heal at all. Free switches of skin from other persons or from the child's parents, while appearing to heal initially, were extruded after a period of two to six weeks.

Because of the failure of such homotransplants advantage may be taken of the characteristic properties of peritoneum. Kamieński has used the peritoneum obtained in operations on other persons, adults or children, for hernia, hydrocele of the testicle or funiculus spermaticus. He transplanted the peritoneal tissue several minutes after it was obtained or after being preserved in physiologic salt solution with the addition of penicillin and kept in refrigeration for 20 to 30 hours. The flaps of peritoneum thus obtained were applied to the granular surface. He also used a peritoneal graft on fresh wounds as well as on granulated wounds. His results were satisfactory in 20 children and 13 adults. The most favorable dressing after peritoneal application appears to be moist gauze dressing soaked in physiologic saline solution.

## Management of Peritoneal Grafting

Although the omentum is constantly at hand before the abdominal surgeon, even in his way, as pointed out by Freeman (65), there seems to be a general failure to recognize the important surgical uses to which it may be put, and especially is this true of free omental graft. A well known function of the omentum is its almost intelligent tendency to seek out and attach itself to raw or inflamed surfaces, wrapping itself around them in such a way as to afford a maximum of protection. This marked tendency of the intact omentum to adhere to its surrounding is also possessed by free omental graft, which may always be transplanted with great certainty except in the presence of actual suppuration. When this fact is truly appreciated the way is open to a variety of useful and even life-saving plastic procedures, such as the replacement of lost portions of peritoneum, the prevention of adhesions, the strengthening of suture lines, the occlusion of the pylorus or of the intestine, and the control of hemorrhage.

When replacement of lost portions of peritoneum cannot be carried out with peritoneum itself by means of flaps, folds, or convenient transplant, an omental graft from any desirable site may be employed. The necessity for such grafting may arise anywhere in the abdomen and the covering of a large or even a small raw surface may sometimes prevent subsequent complication due to inflammation or adhesions.

The advantage of using a free graft rather than an attached portion of omentum is obvious because the attached omentum may give rise to entangling bands or to injurious traction upon the colon, the duodenum, or stomach. In addition when the omentum is permanently attached to a certain spot, the action of that portion of omentum is manifestly prevented in other areas of the abdomen where it might be urgently required.

The potentiality of producing intestinal obstructions from the use of an attached transplant of omentum should be emphasized. Intestinal obstruction may occur in one of two ways: the more common, the volvulus-like loop of small bowel becomes twisted about the omental pedicle, or the contracted wrapping of a greater omentum often seen in complete and chronic obstruction of the terminal ileum or in the region of the ileocecal valve. Binnie warns against the possibility of internal hernia occurring from the use of attached peritoneal transplants.

Care should be exercised in removing no more omentum than is needed but the flaps must be more than sufficient to cover the wound completely. On an attached peritoneal or omental graft the pedicle should not be puckered by ligation. The free border of the omentum should be used to avoid large vessels and the thinnest and most vascular area of omentum available should be selected.

The graft should be handled mainly with

are not placed under the ureter proximal to the side of the necrosing suture The intestinal muscles are closed over the ureter on the side of the necrosing suture and distally by silk mattress sutures which include both edges of the intestinal muscle and pass under the ureter Folds of parietal peritoneum remain between the approximated edges of the intestinal wall The field is peritonealized. The right ureterointestinal anastomosis is performed first and the left in two or three weeks

The peritoneal reaction prevents a leakage and tends to localize and absorb postoperative infection. The physiologic character of the ureter is preserved by deferring ureteral section and by implantation of the ureter with the nerves blood vessels and parietal peritoneum intact Stricture in the proximal segment is unlikely since the ureter is anchored in the intestinal incision by sutures in the parietal peritoneum. The probability of traction of the ureter is minimized by adhesions between the peritoneum and the intestinal submucosa Obstruction of the small intestine after this procedure appears unlikely

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The graft should be handled mainly with

gloved hand and smooth forceps should be used. After removal the graft should be kept between moist, warm, normal saline-soaked laparotomy sponges until utilized.

The graft should be carefully sutured in place with fine silk or catgut, and the transplant must extend beyond the raw surface. Sutures, preferably 00000 silk or catgut with an atraumatic needle, are interrupted every 2 to 3 cm. and the graft is placed so as to overlie the area of trauma. McGehee and his coworkers (75) agree that this precaution has two advantages: 1) to cover the area of trauma completely, and 2) to allow for tucking or rolling the graft edge. Tension of the graft should be avoided to guard against tearing out the suture in gastrointestinal surgery, to interfere as little as possible with peristalsis during the preparatory stage, and to allow for swelling and edema normally accompanying repair. The raw edge of the great omentum should be turned under and sutured and should not be left in a thick mass to form undesirable adhesions. Success in the use of omental grafts is more likely to follow sharp dissection, a clean field, absolute hemostasis, prompt transfer and accurate suture of the graft in its new location. Free omental grafts then become vascularized, remain alive, become adherent to the underlying attached structure, prevent adhesions to surrounding organs, and at times even remain free from surface adhesions and the presence of pus. Such omental grafts are hemostatic, aid in preserving peristalsis by prevention of crippling and immobilizing adhesions, and they strengthen the weak suture line and resist infection.

When peritoneum is about to be transplanted to any region outside the peritoneal cavity, such as the conjunctiva, the dura, the buccal area, the mouth, tendons, nerves and articular capsules, it is advisable for the patient to be examined preoperatively by the abdominal surgeon, who later removes the omentum or the peritoneum. At the time of operation the abdomen and the recipient parts should be prepared simultaneously. The members of the surgical team obtain the piece of omentum or peritoneum through a small muscle-splitting incision and lay it on a sterile gauze sponge which has been soaked in saline. The gauze with the peritoneum on it is then placed in a sterile covered beaker until needed.

## BEHAVIOR OF PERITONEAL GRAFT

The cell survival theory has been so comprehensively presented by Dr. Peer (see chapter 1) that there is little to add to further the understanding of the fate of transplanted tissues. There are, however, certain observations regarding the behavior of peritoneal grafts as they react to the host environment which would

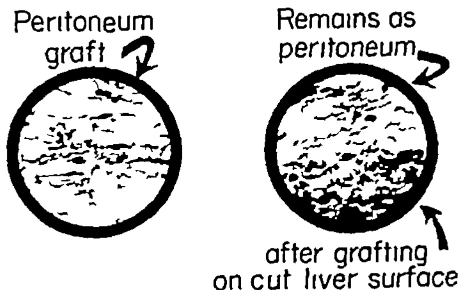



FIG. 25. In the left microphotograph is a section of peritoneum used as graft to cover the raw surface of liver. The right microphotograph is a section of the same peritoneal graft one year later. Note viable peritoneum in upper half of the section covering viable hepatic cells.

confirm our limited knowledge of this debatable subject.

The survival of the mesothelial cells of peritoneum in autogenous peritoneal graft varies according to the recipient area, within or outside the peritoneal cavity according to the environment of the transplanted tissue and according to which surface of the peritoneum the internal or external has been applied to the site of grafting The experimental work of Lexer and Loewy show that the peritoneal transplant does not survive when it is grafted outside the peritoneal cavity

A peritoneal graft on a defect of the pericardium, as Lexer ascertained does not survive. As he stated the transplanted peritoneum becomes adherent to the pericardium by fibrous material and thereby closes the pericardial cavity Blood vessels, round cells, and fibroblasts later invade the deteriorating transplanted peritoneum and replace it. Only a few cells of the transplanted tissue survive and take part in covering the experimental defect of the pericardium. Remnants of the transplant are present in the form of pale, swollen islands of fibrous tissue containing no nuclei

When a section of transplanted peritoneum on the ureter was examined histologically by Lodigani and Esposti (83) they were not able to recognize any peritoneal tissue. Bier (68) in 1918 thought that the endothelium does not survive but rather that it deteriorates rapidly At no time did he observe real takes but he always noted necrosis.

A free transplant of omentum in the peritoneal cavity may remain apparently viable for as long as one or two years and may persist as peritoneum. In many instances however after a few weeks the transplant is reduced to an almost scar-like tissue Brock Ducasting and Reilly (100) established the fact that free omental graft survives while preserving its specific character and that histologically there is a remarkable persistence of the endothelium.

The author's experience with peritoneal grafts on the raw surface of the liver examined at intervals from one week to two years revealed that *the peritoneum remained apparently viable for as long as two years* though in some instances the graft was transformed to scar tissue. A section of a portion of peritoneum used as a graft to cover a raw surface of the liver is shown in the left microphotograph of figure 225. In the right microphotograph of this figure, a section of the same peritoneal graft taken one year later is presented. In the upper half of the section viable peritoneum is seen covering viable hepatic cells.

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PART XI

# *Tumors*

# Transplantation of Tumors

CHAPTER 16

E. J. EICHWALD
with the assistance of N. L. WHEELER

The purpose of this chapter is to acquaint the uninitiated with the essential features of tumor transplantation. It is not intended to be a detailed account of the historical, genetic and immunologic aspects of tumor transplantation. Instead emphasis is placed on general biologic phenomena, particularly on the similarities and dissimilarities in the behavior of transplanted normal and transplanted tumor cells.

The material is grouped into three main sections. The first deals with the problems confronting the graft in the interval between removal from its site of origin and inoculation into the host. A discussion of storage and preservation of tumor grafts is presented in this section. The second section treats events occurring from the time the graft is inoculated to the time the immune response of the host comes into full play. This section includes a review of grafting techniques, vascularization phenomena, the problem of natural resistance and transplantation of human tumors. The third section is a discussion of genetic and immunologic factors that influence the response to and decide the ultimate fate of the graft.

## INTRODUCTION

The first published account of tumor transplantation is that of Hanau in 1889. In the relatively short time that has since elapsed a literature has accumulated which exceeds in volume, diversity and scientific quality the bibliographies of the transplantation of any other tissue. One reason for this rapid accumulation of literature is the wide appeal of the subject—it appeals to the cancer therapist, the immunologist, the biochemist, the geneticist, and others. Another reason is the convenience of tumor transplantation as a scientific tool—no special skills are required although they may be applied; the results are readily recognized and the end points are usually sharp. The relatively brief and predictable duration of experiments facilitates planning of investigations.

Transplantable tumors are readily obtained, either from cancer laboratories or by development from a spontaneous or induced tumor arising in a colony. In consequence there is a vast array of transplantable tumors in use all over the world. In a recently published manual (1) Dunham and Stewart list many of these transplantable tumors, their accessibility, strain specificity, pathogenicity, mode of growth, morphology and bibliographic references. This manual is of great help to persons who wish to start or expand a study with experimental tumors.

### Transplantability of Normal and Neoplastic Tissue

Many textbooks dealing with the subject of cancer convey the impression that transplantability is a property which sets tumor tissue apart from normal tissue. This impression holds an element of truth. A successfully transplanted fragment of tumor tissue may, in a relatively short time, attain a size comparable to the mass from which it was taken, while a successfully transplanted fragment of kidney will not do so. The fragment of kidney may be very difficult to find. This difference of growth is explained by the fact that malignant tissue naturally increases in size while adult renal tissue does not. There

fore it is not a difference in transplantability *per se*.

Another consideration gives emphasis to this difference. It will be pointed out later that the great majority of cells of a tumor graft succumb even if the grafting procedure is successful. The few surviving cells give rise to the tumor. If one assumes that the majority of cells of a transplant of kidney tissue also succumb, it would be difficult indeed to find the surviving cells. Nevertheless, biologically speaking, both tissues have been successfully transplanted.

Transplantability is influenced by the ability of the graft to acquire an adequate food supply, an ability dependent on the organization of its vasculature. Large grafts having a highly organized vasculature, such as transplanted kidneys, must establish anastomoses with the vessels of the host in a short time. Whenever adequate anastomoses do not occur spontaneously, the problem resolves into one of surgical technique. Because tumor tissue does not have a highly organized vasculature and a few surviving cells needed for a graft to succeed can be nourished by adjacent tissue fluid of the host, the problem of surgical technique is negligible. As the graft increases in size, a new vascular system is established.

### Criteria of Successful Transplantation

The difficulties in defining the success or "take" of a transplant are in part due to failure to consider differences in cell behavior as far as growth is concerned. Handler has compiled the definitions of many terms used in tissue transplantation as proposed by various outstanding investigators (2). These investigators, all active in tumor transplantation, have established criteria of success which vary considerably. Many of these criteria are rigidly specific and carry the earmark of preoccupation with malignant tissue or even with one type of malignant tissue. An example of such a criterion is "increase of the graft to a multiple of its original size"—a criterion not applicable to benign tumors and in disregard of the fact that a large portion of a successfully transplanted tumor may be necrotic. Another example is "vascularization of the graft"—a criterion not applicable to ascites tumors or leukemia.

Failure to define accurately what is considered a successful transplantation has caused much confusion. When tumors are successfully transplanted to a host of a different species (using the eye or the brain as sites), the transplanted tumors usually grow *for a limited period of time*. As will be pointed out later, their growth is terminated by the immune response of the host. These "successes" are failures from the point of view of investigators engaged in homotransplantation of tumors who insist on progressive growth of the transplanted tumor followed by death of the host as the criterion of success. It is mandatory that reports in this field carry a definition of the term "success"; also, it would be well if ambiguous terms such as "take" were avoided.

## THE GRAFT BEFORE TRANSPLANTATION

The first step in tissue transplantation is the removal of the graft from its site of origin. When the graft is removed its blood supply is severed and its metabolic and respiratory needs cannot be met; catabolic products accumulate which, prior to the graft's removal, were carried off through the intact venous and lymphatic channels. Lack of oxygen, the most severe and acute deficiency, causes extensive damage and kills many cells.

The magnitude of the injury caused by anoxia incidental to removing the graft is not always fully appreciated. It comes into sharper focus when one considers that anoxia is the most common cause of necrosis. Sudden interruption of the blood supply to a tissue usually leads to infarction. Tissue death in infarction occurs in spite of the fact that the anoxic tissue is not removed from its natural site and is not exposed to dehydration, changes in temperature, or other harmful factors.

Anoxia is only one of the tribulations befalling the graft on removal from its site of origin. Unless the graft is maintained in a balanced physiologic medium, water evaporates from its surface, particularly the cut surfaces, and hypertonicity of the extracellular fluid occurs. Histologic study of such a graft prior to inoculation shows pyknosis of the peripheral cells while the cells in the center appear unaltered (fig. 220). Blood oozing from the severed capillaries forms an irregular film or fibrin over part of the graft surface. Although this fibrin may be of some use in anchoring the graft to a new site, it possibly hinders full utilization of oxygen in a new host environment.

### Storage of Grafts

If a graft is to survive after removal from its original site it must be either transplanted to a susceptible host, placed in a tissue culture where its metabolic and respiratory needs are fairly adequately provided for, or stored at a low temperature to decrease its metabolism.

When stored at room temperature tumor tissue rapidly becomes deficient in necessary metabolites and accumulates harmful catabolites. As a result the tissue survives briefly—usually less than 24 hours. Lower temperatures, however slow the metabolic and enzymatic activities of the tissue and its survival period is increased in proportion to the degree of cooling. When kept at temperatures of conventional refrigeration—slightly above freezing—tumor tissue can survive several weeks of storage. Before storing such tissue must be placed in a physiologic medium, such as physiologic salt solution or Tyrode's solution, and properly sealed to prevent dehydration and hypertonicity in the peripheral portions of the graft.

When survival of a graft beyond a few weeks is desired the temperature has to be lowered further Freezing, with or without subsequent drying, serves this purpose, although both freezing and drying represent dangers of a new kind for the stored tissue. These dangers have been extensively studied (3 4) and generally are grouped in the following categories (1) thermal shock—direct damage to the tissue from the cold (2) crystallization extra and intra cellular within the tissue and melting of the crystals on re-warming—both events believed to damage the cells mechanically (3) changes in electrolyte concentration—when the extracellular fluid begins to freeze it crystallizes out as water (5) leaving the not yet frozen fluid in a hypertonic state that dehydrates and damages the cells.

There has been much discussion over the merits of rapid versus slow freezing While an abrupt drop in temperature appears more harmful to cells highly sensitive to thermal shock, for certain other tissues it has advantages. It is believed that during rapid freezing the temperature level at which crystallization of water occurs is passed so quickly that there is no time for crystal formation or at least that crystallization is incomplete. Under these circumstances freezing without crystallization (vitrification) occurs the harmful mechanical effects of crystal formation are avoided or are at least decreased

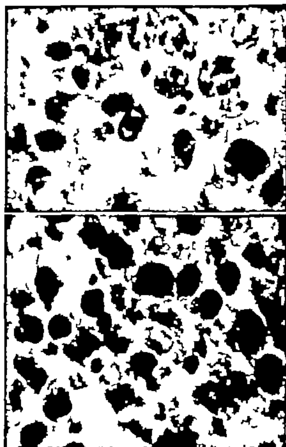


FIG 226 Graft of mouse tumor E0771 two hours after removal from donor kept dry and at room temperature *Above* Center of graft showing well preserved cellular detail with mitotic activity *Below* Periphery of graft showing degeneration of marginal cells

The proper preservation fluid is important in preventing or buffering the effects of freezing Of the fluids studied, glycerol has become the best known. It diffuses rapidly through extracellular fluid, cell membranes, and intracellular fluid and protects the stored cells against some of the harmful effects of electrolyte disturbance. There are conflicting reports on permanent damage to tumor tissue caused by storage. It has been observed that the latent period between inoculation of the graft and its ready palpability is prolonged, that the incidence of successful transplantation is lowered and that such transplants regress after an initial period of growth.

In general, it is surprising how much punishment stored tumor cells can take (6) in surviving extensive freezing and drying A mince of mouse Sarcoma 37 exposed to −190°C for periods up to 27 days, was noted to remain viable (7) Inoculations after thawing were 100 per cent successful, and only a moderate increase in the latent period between inoculation and palpability

was noted. In a small percentage of inoculations C3H Sarcoma, which was stored in a glucose solution at −79 C for as long as 25 days and then dried to dust in vacuo at a constant temperature of −25 C and reconstituted gave rise to tumors. Successful transplantation of tumor tissue stored for as long as two years has been reported.

These studies are of interest to proponents of the theory of a virus etiology of cancer. The preceding observations could lend themselves to the argument that after such long periods of storage a non-cellular agent of an infectious nature was transferred instead of viable cells and that the tumor did not arise from surviving tumor cells but from a reaction of the host tissue to the infectious agent. There is good evidence against such an assumption. As an example, morphologically normal tumor cells can be seen in the reconstituted frozen-dried material. Additional proof that a tumor does not arise from the host but develops from the graft even after prolonged storage has been adduced by means of an ingenious experiment. A graft was embedded in a plasma clot and the clot containing the stored graft was then transplanted, partially isolating the graft from host tissue. Microscopic study soon after transplantation revealed clusters of growing tumor cells within the plasma clot but no proliferative changes in the adjacent host tissue (8).

## THE GRAFT AFTER TRANSPLANTATION

### Establishment of Food Supply and Drainage

The events to be described occur soon after transplantation of a solid tumor fragment and when a solid tumor results. Events differ when the tumor grows in a disseminated manner as do ascites tumors and leukemia. Events also differ when a cell suspension is inoculated.

Placing the graft in a host promises relief from its two chief predicaments: lack of metabolites, including oxygen, and accumulation of catabolites. Whatever host and whatever site are used, the surroundings are usually of body temperature, gently flowing, and contain a small but steady supply of oxygen, amino acid, glucose, and other metabolites. The surrounding fluid will reestablish isotonicity of the marginal portion, dissolve any fibrin, and wash away catabolites from the surface and from the crevices of the graft, clearing the way for further washing out of backed-up waste products. These events facilitate the survival of a few fragments of the outer shell. Soon after transplantation the marginal portion of a graft appears healthy while the center is necrotic (fig. 227). Growth of the graft beyond mere survival depends on the development of a vasculature to help supply metabolites and eliminate catabolites. Vascularization accomplished by the growth of host capillaries into the graft, establishing anastomoses with any vascular channels of the graft, takes place within a few days and has been reported as early as the first day after transplantation (9). Most studies in this field have been conducted in Algire's laboratory, where transparent chambers have been used to much advantage (10). This technique has thrown light on the early events following transplantation of tumor and normal tissues and has been helpful in analyzing and differentiating manifestations of host immunity, which will be discussed later in detail.

For reasons not entirely clear the presence of an ample supply of metabolites and adequate drainage do not assure survival of cells after transplantation. An example is the fate of metastasized tumor cells. (Metastasis, in effect, is spontaneous autotransplantation of tumor tissue.) Not infrequently tumor emboli thrown into the pulmonary circulation adhere to the endothelium of the vessels. Instead of giving rise to metastases they degenerate and become necrotic (11) even though the food supply and drainage appear to be adequate.

### Regeneration of the Graft

The basic principles of graft survival at this state are the same whatever the transplanted tissue may be. Even if undisturbed in their natural site all cells have a limited life span. The remaining viable cells of a graft therefore have to multiply if any portion of it is to survive. A tissue with a short cellular life span, a high cell turnover, and a high metabolic rate (tumor, blood, fetal tissue) readily compensates for a substantial loss of cells provided a small number survive from which regeneration may occur. This provision is crucial. Such tissue is more endangered by temporary anoxia incidental to transplantation than a tissue with a long cellular life span, a low turnover of cells, and a low metabolic rate (bone, fibrous tissue, cartilage). However, once portions of these slowly regeneratin

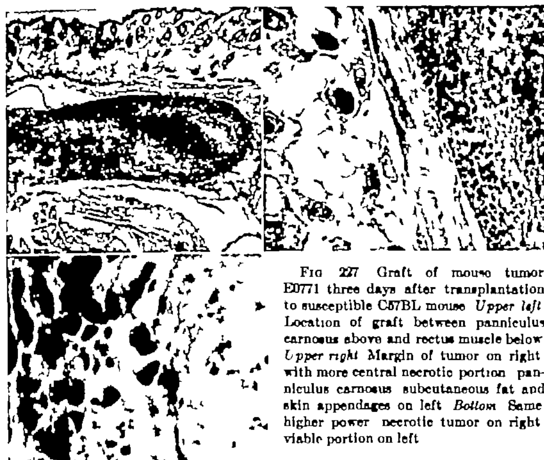


FIG 227 Graft of mouse tumor E0771 three days after transplantation to susceptible C57BL mouse *Upper left* Location of graft between panniculus carnosus above and rectus muscle below *Upper right* Margin of tumor on right with more central necrotic portion panniculus carnosus subcutaneous fat and skin appendages on left *Bottom* Same higher power necrotic tumor on right viable portion on left

tissues are destroyed the task of recuperating is arduous.

The basic problem faced by the neoplastic cell after transplantation, then, is similar to that faced by the normal cell. Both are threatened by anoxia, and appreciable numbers of both succumb Differences in the fate immediately after transplantation are due less to profound biologic differences between normal and neoplastic cells *per se* than to differences in cell turnover life span, and metabolic rate It so happens that most tumor cells live a shorter time than most normal tissue cells. Hence transplants of most tumor tissues are better able to halt and even overcome an initial loss of cells than normal tissues. This does not apply to all tumor tissues. Many slowly growing highly differentiated tumors are less readily transplanted than some non-tumorous structures such as embryonal tissues

## Natural Resistance

It would appear from the preceding paragraphs that all is well with a graft if adequate nutriment and drainage are provided. However the graft is confronted with inimical non-specific host factors which are present at the time of inoculation and form a significant part of the host's defense against foreign tissue These factors represent the natural resistance of the host. Presumably antibodies in the intercellular fluid of the hosts are involved Host cells—polymorphonuclear leukocytes lymphocytes histocytes and others—could act directly against the foreign substance or release compounds which affect the foreign cells adversely The natural resistance to the graft may have been inherited as part of a non-specific primary defense mechanism. It may be the result of previous contacts with a variety of foreign cells or proteins

The variation in natural resistance at different host sites is a circumstance utilized extensively by investigators who wish to overcome host barriers as in the transplantation of human tumors to a different mammalian species, or transplantation of tissue from one zoological class to another *e.g.*, chicken to guinea pig (12) Sites of low natural resistance which have been used extensively for the heterologous transplantation of tumors are the anterior chamber of the eye (13) the brain of various species (14) and the cheek pouch of hamsters (15 16) Other sites which have been used successfully are muscle and testicular tissue, cornea, the subdural

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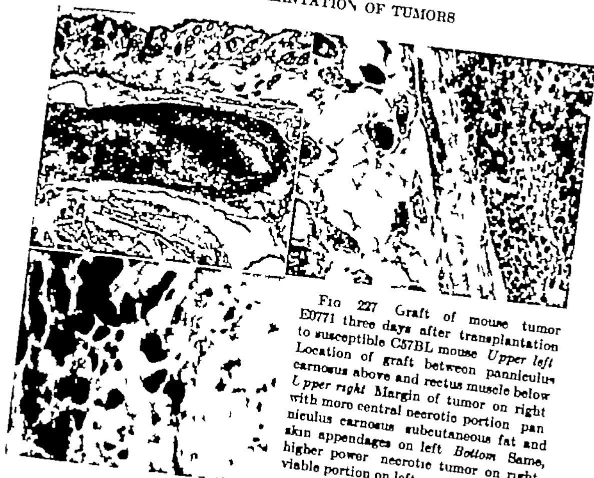


FIG 227 Graft of mouse tumor E0771 three days after transplantation to susceptible C57BL mouse *Upper left* Location of graft between panniculus carnosus above and rectus muscle below *Upper right* Margin of tumor on right with more central necrotic portion panniculus carnosus subcutaneous fat and skin appendages on left *Bottom* Same, higher power necrotic tumor on right viable portion on left

tissues are destroyed, the task of recuperating is arduous.

The basic problem faced by the neoplastic cell after transplantation, then is similar to that faced by the normal cell Both are threatened by anoxia, and appreciable numbers of both succumb. Differences in the fate immediately after transplantation are due less to profound biologic differences between normal and neoplastic cells *per se* than to differences in cell turnover life span, and metabolic rate It so happens that most tumor cells live a shorter time than most normal tissue cells. Hence, transplants of most tumor tissues are better able to halt and even overcome an initial loss of cells than normal tissues. This does not apply to all tumor tissues. Many slowly growing highly differentiated tumors are less readily transplanted than some non-tumorous structures such as embryonal tissues.

## Natural Resistance

It would appear from the preceding paragraphs that all is well with a graft if adequate nutriment and drainage are provided. However the graft is confronted with inimical non-specific host factors which are present at the time of inoculation and form a significant part of the host's defense against foreign tissue These factors represent the natural resistance of the host. Presumably antibodies in the intercellular fluid of the hosts are involved. Host cells—polymorphonuclear leukocytes, lymphocytes, histocytes and others—could act directly against the foreign substance or release compounds which affect the foreign cells adversely The natural resistance to the graft may have been inherited as part of a non-specific primary defense mechanism. It may be the result of previous contacts with a variety of foreign cells or proteins

The variation in natural resistance at different host sites is a circumstance utilized extensively by investigators who wish to overcome host barriers as in the transplantation of human tumors to a different mammalian species or transplantation of tissue from one zoological class to another *e.g* chicken to guinea pig (12) Sites of low natural resistance which have been used extensively for the heterologous transplantation of tumors are the anterior chamber of the eye (13) the brain of various species (14) and the cheek pouch of hamsters (15, 16) Other sites which have been used successfully are muscle and testicular tissue cornea the

space of the brain and spinal cord and the pregnant uterus. It is of interest that immune responses in these sites are delayed and weak (17). Although several theories have been advanced to explain the suitability of such sites, none has been generally accepted. To state that the natural resistance in such sites is low begs the question since neither is it clear what natural resistance is, nor is it known *why* natural resistance is low in such sites.

### Transplantation of Human Tumors

Homotransplantation of human tumors has not been practised extensively nor, in general, successfully because of the immense diversity of human genetic characteristics. There are a few reports of successful transfers, presumably transplacental, of malignant melanomas from mothers to newborn infants (18). These instances could be explained by the serologic immaturity of newborn individuals which prevents a successful combat against the foreign tissue, a phenomenon well known in related fields (19).

Heterotransplantation of human tumors—a field of study greatly stimulated by the work of Greene (21) has been practised extensively and often successfully since sites of low natural resistance (eye, brain) can be utilized in lower species. The criteria for "success" are less stringent than in homotransplantation. Initial growth of a tumor in a site of low natural resistance is considered a success even though in most instances the immune response of the host will cause ultimate regression.

Most investigators who have transplanted human tumors have done so for reasons of pure biologic curiosity. Many successes have been reported, but there have been even more failures. Investigators who have published large series report success in from 12 to 85 per cent of human tumors (16, 20–23). Sarcomas and bronchogenic carcinomas have often been found more transplantable than breast carcinomas; lymphomas are not readily transplanted.

Heterotransplantation of human tumors may prove to be of practical significance since a variety of therapeutic agents can be applied to such transplants just as bacterial colonies are exposed to a variety of antibiotics to test sensitivity. This field of endeavor has been greatly aided by Toolan (24) (further referred to below) whose studies have established permanently transplantable human tumors that are maintained in irradiated and/or cortisonized heterologous hosts. Some of these tumors are already being used in large scale pharmaceutical screening.

### Methods of Tumor Transplantation

The most common method of tumor transplantation is the transfer of a solid fragment of viable tumor tissue to the subcutaneous tissue of a host. Conventional principles of asepsis should be observed although under ordinary circumstances a reasonable degree of cleanliness suffices. The donor of the tumor tissue is anesthetized or killed. If the donor is killed, the tissue to be grafted should be removed as soon as possible since its viability decreases rapidly even at low temperatures if left in the dead donor. Ulceration of the donor skin, a common event in advanced stages of growth of subcutaneous tumors, is an unfavorable factor because of bacterial contamination, but slight ulceration of the donor skin usually does not prevent successful transplantation (25).

The method described is one of many since each investigator develops his own variations. The skin around the tumor is incised, and reflected with the tumor attached. Depending on the extent to which the tumor has invaded, portions of the underlying normal structures (muscle, fascia) will adhere to its surface. Care should be taken to remove them. When the denuded tumor is incised, it usually reveals a shell of semi-translucent viable tissue and a massively necrotic core. A slight admixture of necrotic tissue does not stand in the way of a successful transfer. When one intends to inoculate a fairly large number of animals, the viable portions of the tumor should be removed and set aside in a Petri dish humidified by enclosing a moist sponge or a wad of cotton to prevent dehydration and stickiness of the tumor surface. The tumor is inoculated with a trocar prepared from a lumbar puncture needle (gauges 15 to 21) whose tip is ground down a few millimeters so that the fully inserted plunger will protrude over the bevel and assist in disengaging the graft.* A small fragment of tumor is placed on the bevel of the needle and sucked into the barrel by a backward motion of the plunger. Sterile Vaseline applied to the proximal portion of the plunger provides an airtight fit useful for drawing the

* A special trocar designed for this purpose is commercially available (Becton, Dickinson & Co. No. 468 LRT).

graft into the barrel.* The loaded trocar is inserted through a nick in the host's skin the plunger pushed forward and the tip of the trocar and plunger are pinched from the outside to help disengage the graft.

In most instances this technique places the graft in a "subpannicular" location (fig. 227) — the loose space between the panniculus carnosus and the body wall. The trocar also can be inserted into the true subcutaneous site (27)— the restricted space between the skin and the panniculus. Other transplantation sites require some modification in technique. For intracranial inoculation of mice a smaller and shorter needle is used to pierce the skull in larger animals burr holes or bone flaps are needed.

For large numbers of animals suspensions of tumor cells have the advantage of faster administration, a higher degree of uniformity and less contamination. With proper dosage concentration and size of particle the results are the same as with solid grafts. Suspensions can be prepared by first mincing portions of tumor with fine scissors. To decrease further the size of the particles, the mince, properly diluted is passed through layers of gauze, needles of decreasing caliber or a special cytosieve devised by Snell (28) In the author's laboratory a Ten Broeck tissue grinder (A. H. Thomas & Co) is used in which the mince is ground with a counted number of slow gentle strokes and rotations. It is then passed through a 'coarse' fritted glass filter (maximum pore size 40 microns) The manner of homogenization is important as the viability of some tumors decreases rapidly after such handling. The size of a graft— within the general order of magnitude practicable for the inoculation of solid tissues—is immaterial. If tumor cell suspensions are used instead of solid grafts, the outcome may be jeopardized by decreasing the concentration and quantity of the suspension.

The number of cells required for successful inoculation varies from tumor to tumor and is influenced by the size of the particle in the suspension, the degree of dispersion and the viability of cells. That tumors can arise from a very small number of cells under favorable circumstances can be demonstrated in a simple manner by using the anterior chamber technique which permits ready observation of an inoculum. In a susceptible strain of mice grafts placed in the eye and then removed within seconds invariably give rise to tumors (29) which presumably develop from cells rubbed off the graft during insertion and removal.

It has been reported that 25 cells are needed to transplant C3H Sarcoma successfully and 1641 cells are needed to transplant Sarcoma 37 successfully to 50 per cent of susceptible recipients (30) By use of a micromanipulator leukemias have been transplanted by single cells (31)

On first thought, it would seem that every tumor would be transplantable with a single cell. In hospitable surroundings a single healthy cell could multiply and in due time give rise to a sizable tumor But this is not the usual course of events. Because of the damage to the graft cells during transfer each individual cell has only a slight chance to survive.

## ULTIMATE FATE OF GRAFTS

### Genetic Aspects

In the early history of tumor transplantation experimental results were not easily reproducible. A tumor transplanted from one rat to another or from one mouse to another would at times succeed and at times fail. Transfers within the same litter succeeded more frequently than transfers between less closely related animals. However it was not uncommon to have transfers between litter mates fail, while transfers to apparently less closely related animals succeeded. Tyzzer Little Snell, and other workers at the Roscoe B Jackson Memorial Laboratory at Bar Harbor Maine, have brought order to this apparent confusion and raised studies of tumor transplantation to a scientific level. By producing genetically pure, or homozygous, strains of mice by continuous brother-sister matings, they created a superb biologic tool with which genetic relationships of desired purity or calculated impurity could be created.

It is now generally accepted that the fate of the graft is determined primarily by the genetic relationship of the recipient to the graft. When this relationship is close, as in the case of inbred strains, identical twins or autotransplantation, the graft will succeed provided it survives the trauma of transfer When the relationship is

* A semi-automatic needle has been devised in the author's laboratory which delivers grafts of uniform size and shape eliminates excess handling of the tissue and obviates tedious and time consuming reloading (26)

very distant, as between animals of different classes the ultimate and very likely the initial fate of the graft will be rejection.

Rejection of a tumor graft almost certainly is effected by an immune response of the host to the graft This response is forthcoming whenever *the graft carries certain antigens, or factors,* which are absent in, and therefore alien to the host. The absence or presence of these factors govern the fate of the transplant in a manner comparable to Rh immunization.* Since the factors are concerned with the compatibility or incompatibility of various tissues, they are called histocompatibility factors. They are inherited as dominant genes (similar to the ABO factors of human blood) and are also referred to as *histocompatibility genes*

Since brother-sister matings for many generations (done so far almost exclusively with mice) finally result in a progeny that is genetically identical or almost identical ("inbred strains") there are no (noteworthy) differences of histocompatibility genes within such progeny In consequence tumors arising in an inbred strain are transplantable within that strain. It follows then that tumors are also successfully trans*plantable to all mice carrying the histocompatibility genes* of the tumor For example $F_1$ hybrid mice of two pure strains (AA BB) carry the histocompatibility genes of both parental strains (AB) and hence are susceptible to tumors of either parental strain. However neither parent strain is susceptible to tumors arising in the $F_1$ hybrid because cells of the $F_1$ hybrid (AB) carry the histocompatibility genes of *both* parental strains and therefore are in part alien or antigenic to each parent strain ("$F_1$ principle")

Tumors of an inbred strain are also transplantable to a certain percentage of $F_2$ hybrids and "unrelated backcrosses" (the progeny of an $F_1$ hybrid and a pure parent of the other strain) Susceptibility of these mice depends on whether or not they contain the histocompatibility genes of the tumor This is a matter of chance the magnitude of the chance depending on the number of histocompatibility genes involved. If the tumor and grandparental strain have one pair of histocompatibility genes (AA) the chance of an $F_2$ hybrid carrying the same gene is high (75 per cent) there being four different possible gene combinations (AB BA, AA, BB) three of which carry A. Another 75 per cent, in part overlapping will be susceptible to tumors of the B strain. Only 50 per cent will be susceptible to tumors from either strain.

The chances of an $F_2$ mouse carrying all histocompatibility genes of a pure grandparent drop rapidly when more than one pair of histocompatibility genes is involved. It is readily calculated that the percentages of successful tumor growth decrease as follows 3.1 per cent with 5 0.1 per cent with 10 0.002 per cent with 15 pairs of genes, *et cetera*. Inversely the number of genes involved can be estimated on the basis of such percentages Somewhat similar percentage values apply to unrelated backcrosses.

Snell and his coworkers have analyzed several of the more important histocompatibility loci (*H 1 H 2 H-3*) their relative strength and distribution in various mouse strains (32 33) Numerous alleles, mainly of the *H-2* gene have been described and characterized by lettered superscripts. These studies have been done with the *in vivo* method of tumor transplantation and the *in vitro* method of hemagglutination, also utilizing absorption techniques.

The results of tumor transplantation are not always as clear-cut as it may appear from the above account. Not infrequently tumors are not transplantable within their strain of origin, particularly in the first transplant generation, and occasionally they are transplantable to mice of other strains. Also the percentages of successful transplantation to $F_2$ hybrid or unrelated backcrosses at times do not "fit" any number of genes, as calculations would suggest. There are several possible explanations for such results. Aside from previously discussed circumstances which may interfere with the initial acceptance of the graft—improper technique unsuitable host sites, or high sensitivity to the trauma of transfer (as will be the case with cells having a short life span)—there are two main factors which may interfere with the simple arithmetic formulas described One is the balance between the

* If the host (Rh positive mother) carries the same factor( ) a the graft (Rh positive fetus ) the host will not produce an immune response (*no agglutination of Rh positive cell* ) (Such an immune response would damage not only the graft but also the host ) If the host (Rh negative mother ) does not carry the factor( ) of the graft (Rh positive fetus ) an immune response will ensue (formation of anti Rh antibodies ) and the graft (fetal red blood cell ) will be destroyed

strength of the immune response and the sensitivity of the tumor to it. Like other antigens those under the influence of histocompatibility genes vary in strength. A strong antigen will give rise, in a normally reacting host, to a response sufficiently strong to cause rejection of a tumor. A weak antigen may merely retard tumor growth, or may manifest itself only if the host has been specifically sensitized to the antigen. As an example, a fibrosarcoma induced at the author's laboratory in a C57BL male mouse appears to carry a weak histocompatibility gene on its Y (or sex) chromosome: its growth is retarded in C57BL female mice and their hybrids, and occasionally it fails. In properly sensitized C57BL females it always fails to grow (29). The presence of such a weak gene may therefore prevent or modify the growth of a tumor in otherwise susceptible mice. Nor are all tissues equally sensitive to immune responses. A rapidly growing tumor may not be greatly affected by an immune response sufficiently strong to cause rejection of skin; in fact, it may kill a host before the response has attained its full strength. An illustration is the described response that arises on the basis of the Y linked histocompatibility gene. While it will cause mere inhibition of a tumor, it is sufficiently strong to cause rejection of all skin grafts of C57BL males to C57BL females and their female F1 hybrids (34).

The other factor is the possibility that the chromosomal upheaval giving rise to tumor growth involved the histocompatibility genes of the cells from which a tumor arose. A new histocompatibility gene may be acquired or an existing gene modified; in either case the tumor may not be transplantable to mice of the indigenous strain. On the other hand, an existing gene may be lost, and the tumor will become transplantable to previously resistant hosts.

### Immunologic Aspects

A transplanted tumor either grows progressively killing the host, fails to grow because the natural resistance of the host is too strong, or grows initially but ultimately regresses. The geneticist, in general, is not concerned with the question whether resistance to the transplanted tumor was present at the time of transplantation or whether it arose as the result of the transplantation. Both early and late rejections are interpreted as the expression of an immune response which developed because the host lacked in its genetic make-up the histocompatibility factor(s) of the transplant.

Regression of a tumor transplant after an initial period of growth is comparable to the rejection of a skin graft after an initial period of acceptance and is one of many observations which attest to the ability of animals to acquire immunity to transplanted tumors (33, 35–41). Other manifestations of acquired immunity are the deceleration of tumor growth, the shortening of the period of initial growth, and absence of all growth. Susceptible animals can be made resistant by a preceding inoculation of killed tumor tissue, normal tissue, extirpation or strangulation of a growing tumor.

Experiments demonstrating the appearance of antibodies suggested that many of the observations were indeed immune phenomena. Such antibodies have been demonstrated many times since 1911 when Lambert and Hanes observed that cytotoxins adversely affect tumor cells growing in tissue culture. As a rule, antibodies are more readily demonstrated when the genetic gap separating the graft from the host is wide, or, in the language of Leo Loeb, when "the differentials which serve as antigens are coarse."

Complement-fixing antibodies, precipitins, and lymphocytopenia-producing antibodies have been demonstrated. Neutralizing antibodies which interfere with the transplantability of tumor cells also have been demonstrated. Gorer, Kaliss and Snell have studied hemagglutinins which develop following transplantation of a tumor to mice of a resistant strain. These agglutinins clump the red blood cells of mice of the donor strain. Hence it is assumed that the tumor cells carry an antigen, absent in the resistant host, that gives rise to the formation of hemagglutinins (32, 42, 43).

The appearance of circulating antibodies when the phenomena of acquired immunity become manifest permits the inference that the antibodies are instrumental in effecting the destruction of the graft. It is quite likely, however, that any demonstrated antibodies are only *part* of the immune response. Additional antibodies produced by the host may also contribute. It is conceivable that more than one type of antibody is necessary to effect regression of the graft.

Possibly some antibodies are merely by products of an immune response products not utilized in the defense of the host. This may explain why antibodies can occasionally be demonstrated although they appear to exert little influence on the graft That antibodies often cannot be demonstrated when one would expect them to be present is very likely due to the inadequacy of the techniques employed

Studies with diffusion chambers (44) have illuminated the nature of the antibodies involved Diffusion chambers utilize millipore membranes* of known pore diameters which will transmit large molecules but will not transmit cells. A tumor graft will survive in a foreign strain if placed in a diffusion chamber This observation suggests that the direct contact with host cells plays a major role in the rejection of grafts.

The role of the blood vessels in the immune response to a graft has been extensively studied. It is still being debated whether the thrombotic occlusion of host vessels seen after graft rejection is the cause or effect of that rejection. The question has been asked whether a blood supply is needed to elicit and transmit an immune response. There is evidence that a graft does not elicit a strong immune response unless it is vascularized by the host. There also is evidence that such a response is not readily transmitted to a graft unless it is vascularized While this evidence is not clear-cut and appears contradictory at times, the contradictions resolve readily on considering that the *amount* of antigen transmitted from graft to host and the *amount* of antibody transmitted from host to graft are important factors. A well established drainage system will permit more antigenic compounds to enter the host circulation and reach sites and organs of antibody production and a well organized vasculature will provide a rapid and thorough distribution of antibodies to all parts of the graft. Also antigen on its way to pertinent host tissue and antibody on its way to the graft cells have to cross an endothelial barrier and traverse intercellular fluid In vascularized grafts the pathway through intercellular fluid is short In non-vascularized grafts it is long Should the antibodies fail to reach *all* cells of the graft and in sufficient strength small fragments of the graft will survive and recover The effect of the immune response may then be apparent only as a delay in growth. If the dose of antigen transmitted to the host, and the dose of antibody transmitted to the graft approach a critical level, it is likely that any obstacle to the transfer of either antigen or antibody may prevent manifestations of the immune response

It is understandable that phenomena of immunity observed in the study of transplanted tumors were seized by cancer therapists in attempts to immunize patients against cancer to slow the course of growth once cancer had developed, and to prevent recurrence But it soon became apparent that these phenomena observed in association with transplanted tumors were not directed against the cancer as such but against its foreignness, and that the immune phenomena could be elicited by non-cancerous foreign tissue. Spontaneous cancers the object of such therapeutic adventures, were not affected.

### Modifications of Graft and Host

Within certain limitations knowledge of the genetic and immunologic relationship between a host and graft will help to predict the fate of the graft. However it is not unusual for the outcome to differ from the expectation. These unexpected results are of theoretical interest and may be of practical significance since investigating them may help to overcome the immune response of the host to a graft, the greatest obstacle to successful transplantation of normal tissues.

Deviations from the expected fate of a graft are due to spontaneous or induced modifications of either the graft or host Modifications of the graft can express themselves in several ways (45) Unexpected successes or failures are encountered. Changes in growth pattern may occur A carcinoma may become a sarcoma a locally invasive tumor may become a metastasizing tumor a solid tumor may turn into an ascites tumor The growth rate may accelerate an event often termed an increase in "virulence" A tumor may lose specificity and grow in many strains. While some workers have believed, on theoretical ground that such loss of specificity was related to a loss of antigenic properties Feldman and Sachs (43) have demonstrated recently that tumors grow in spite of persistent antigenicity as shown by formation of hemag

* Commercially available from Millipore Filter Corporation 20 Pleasant Street Watertown Mass.

glutinins in the susceptible foreign hosts, an observation similar to that made by Schneweis (46). The reverse also may happen. This capriciousness of transplanted tumors which makes it difficult to compare data from different laboratories, is probably not due to sudden mutations in a uniform population of tumor cells. The cells of a transplanted tumor vary in morphology, chemical constitution and even in number of chromosomes. An abnormal number of chromosomes, aneuploidy and heteroploidy is not uncommon. With standard transplantation procedure, the balance between these various cell types may be assumed to remain relatively constant. By using different transplantation techniques, a different type of host, or host site or following irradiation certain cell types may be lost through selectivity and one type may gain the upper hand. In this fashion a tumor may gradually or suddenly change its characteristics. To a certain extent this modification can be wilfully induced, *e.g* by passing some neoplasms through hybrid hosts (47)

Host factors also may influence the transplanted tumor (48) Starvation may decelerate its growth (49) but in general the tumor holds its own when competing with the host tissues for a limited supply of food Deficiencies in certain specific nutritional factors have been noted to inhibit and even to prevent tumor growth An example of this is pyridoxine deficiency which may retard the growth of lymphosarcoma grafts (50)

A variety of factors known in the laboratory or clinic to interfere with immune responses to antigenic stimuli have been applied to attenuate the host response to transplanted tumors. Some of these are hormonal in nature and concerned with the effects of adrenocortical and pituitary secretion on the host defenses (51 52) Injections of trypan blue India ink, or Thorotrast have been reported to weaken the host response. The rationale behind this procedure is "blockade of the reticuloendothelial system. The injected material stored by these cells is believed to interfere with the production of immune bodies (53)

The use of ionizing irradiation has a similar aim. It has a profound effect on tissues with a high mitotic rate, such as bone marrow and lymph nodes. Since these tissues contribute to the defense of the host, irradiation can be expected to interfere with the immune response (23) As early as 1914 Murphy reported increased susceptibility to transplanted tumors after irradiation of homologous and heterologous hosts (54) Toolan has serially transplanted human tumors over long periods of time in irradiated weanling rats (55)

These examples show that irradiation can interfere with the natural resistance of a host. It also can delay immune responses, *e.g.*, a tumor transplanted to a foreign host will survive longer if the host has been irradiated. However once a host has been specifically immunized against a certain tumor irradiation has little effect. It is not yet clear whether the latter type of immunity differs qualitatively from the other types against which irradiation is effective, or whether the difference is merely a quantitative one.

Similar observations have been reported following administration of ACTH and cortisone. Again, administration of these compounds is more effective in preventing than in abolishing an immunity response (48)

Interference with the host response may follow previous contact of the host with cells or portions of cells from the prospective donor These observations, the opposite of an immunizing effect, have been ascribed to 'enhancing factors or "XYZ factors" present in the tissue preparation injected at the time of previous contact. Hosts treated in this fashion have been described as being conditioned or pretreated.' The first observation—accelerated growth of a rat tumor in susceptible hosts—was reported by Flexner and Jobling in 1907 (56) It occurred when tumor inoculation followed the administration of heat-killed tumor cells. Casey demonstrated that prior treatment with killed tumor cells increased the incidence of progressive growth of the Brown-Pearce tumor of rabbits (57) The enhancing influence was found to be of genetic specificity pretreatment with tumor tissue of the rabbit had no effect on the growth of mouse tumors even pretreatment with tumor tissue of one mouse strain had no effect on the growth of a tumor from a different mouse strain. Kaliss and Snell demonstrated an enhancing effect following injection of lyophilized normal tissue preparations (58) They also studied the physicochemical properties of the factors involved and the significance of dosage. Interestingly the

enhanced state can be transferred passively by injecting antisera from pretreated to untreated mice The effect is observed even if the donor of the antiserum is of a different species *e.g.*, the rabbit (59)

It is debatable whether enhancement is related to phenomena of acquired tolerance as described by Owen, Medawar Billingham, and others (17 60 61) In spite of obvious similarities several investigators believe that these phenomena differ basically tolerance being due to central failure to produce antibody and enhancement, to afferent inhibition inactivation of antigens before they reach sites of antibody formation.

## CONCLUSION

A large volume of data most of them without immediate practical significance, has been accumulated in the field of tumor transplantation. Many investigations of transplantable tumors are an outgrowth of studies on cancer patients it was hoped that tumor transplantation could provide answers to questions confronting the physician who treats cancerous patients. This has proven true only to a limited extent. Most knowledge gained from studies on tumor transplantation is of less significance to the cancer therapist than to the immunologist, the biochemist the geneticist, the experimental morphologist and the surgeon active in replacement therapy As yet practising physicians have barely begun to utilize the scientific armamentarium provided by investigators in tumor transplantation Neither are basic investigators sufficiently aware of the needs of practising physicians It bodes well that in recent years the relationship between these two groups has become closer

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# PART XII

# *Tissue Culture*

CHAPTER 17

# Tissue Culture of Adult Tissue

MARY STEARNS PARSHLEY

## INTRODUCTION

Tissue culture may be defined as the growth or maintenance of cells or organized tissues outside the organism. The development of a method for the direct observation of living cells and tissues removed from systemic influences marked an important advance in the field of biologic research. It made possible for the first time the systematic study of cells isolated from highly differentiated tissues and a correlation of the morphologic and physiologic characteristics of normal tissues. It provided as well a means for the direct observation of the effect on the living cell of an almost unlimited variety of experimental conditions. Important biologic questions, once obscured by the complexity of the tissues involved, were solved. Conspicuous examples are Harrison's direct microscopic observation, in 1907 of the outgrowth of nerve fibers from neuroblasts (1) the demonstration by Burrows, in 1911 of the myogenic nature of the heart beat (2) and the evidence, presented by Carrel in 1914 of the ability of somatic cells to multiply continuously when removed from the organism and isolated in a growth-stimulating environment (3) The strain of fibroblasts which was developed from chick embryonic heart tissue in Carrel's laboratory was maintained for 34 years.

Since Carrel's demonstration tissue culture technique has been applied in limitless ways to the collection of information about the living cell, which is of increasing significance for an understanding of the behavior of human tissues Precise information concerning metabolism and the nutritional requirements of normal tissues has been obtained. The morphologic and functional characteristics of many types of cells from a variety of species have been observed. Many adult human tissues available in the operating room have been used Factors which influence the growth and differentiation of specific properties of cells, their organization, and the influence of one type of cell on another have been studied *in vitro* The development of organs from embryonic anlagen has been followed as well as the regeneration of highly specialized cells of adult tissues, formerly considered incapable of such activity

An early and important application of tissue culture technique was to the study of tumors and to the comparison of the properties of isolated normal and malignant cells. Tumor cells were shown to retain their cancer-producing properties *in vitro* by reinoculation of cultures into animals. Strains of tumor cells were maintained for long periods of time in continuous cultivation, providing material for a study of their metabolic and cytologic characteristics. Normal cells were shown to acquire malignant properties *in vitro* under the influence of carcinogenic agents. Such changes have occurred also spontaneously in cells maintained for long periods in continuous cultivation. The tissue culture technique has been applied to advantage also to the differential diagnosis of human cancer

Tissue cultures have been used extensively to study the effect of drugs on the behavior of cells isolated from systemic influences. Cell reaction can be observed directly—the manner of action of the drug the reversibility of effect, and the level of toxic concentration. A simple and rapid method for the preliminary screening of substances recommended for antibacterial or anti

neoplastic activity is provided. In another important field, that of radiobiology, the tissue culture has been used for some time to study cell sensitivity to radiation. It is now possible to study the action of irradiation on living systems by direct observation of the effects of various rays on cell structures such as the chromosomes.

In the last decade the development of new tissue culture techniques has made possible particularly rapid progress in the field of virology. In this field notably tissue culture has replaced animal experimentation in many areas so that speed and economy have been gained in the development of methods of growing virus, titrating virus and antibody and in the study of the relationship between virus and host at a cellular level as it pertains to the production of vaccines. The method has been applied more recently to the storage of tissue for transplantation and in a few instances also to the testing of its viability at transplantation and to the study of the effect of implantation on both graft and host tissue. As the editor of this book has remarked in volume 1 "The field of tissue culture and its application to a study of the fate of cells in free grafts should develop rapidly because it is an important approach to further knowledge of cell and graft behavior."

## TYPES OF CELLS AND TISSUES GROWN IN TISSUE CULTURE

A variety of plant and animal tissues, both vertebrate and invertebrate have been studied by this method. Although in 1910 Carrel and Burrows (4) described the growth *in vitro* of tissues from adult dogs and cats, the impression has persisted in many texts that only the mesenchymal elements of adult tissue will grow. Actually most types of specialized cells from adult tissues have been grown outside the body including blood cells, endothelial tubules glandular epithelium, fibrous tissues cartilage and bone all types of muscle and the neurons and supporting cells of the nervous system. A list of the literature describing these studies would be too long to be practical. The interested reader however may find such references in *A Bibliography of the Research in Tissue Culture* (5) a catalogue of 15,000 original papers. Embryonic tissues from chick rat and man have been widely used. Tissues of adult fowl rat rabbit guinea pig dog and monkey have been studied also. Today sections of adult human tissues are being used with increasing frequency for tissue culture experiments since the method provides a direct means of observing living human tissue. In the references for the present chapter there are a number of excellent texts (6-9) that describe in detail methods of tissue culture and their application. These methods have become increasingly numerous, complicated and specialized for different types of experimental work.

Depending on the purpose of the experiment the observer has focused on the piece of cultured tissue itself the explant or on the outgrowth of new cells from it or on both. In studies of the development of organ rudiments or the differentiation of some specialized cells *in vitro* the explant is of main interest. Observations are made to a great extent from fixed and stained cultures or from histologic section. Studies of the physiologic activities of explanted organs depend also on observation of the explant in the living state. Surgeons who have adapted the tissue culture method to the preservation of small fragments of tissue for grafting have been interested mainly in preserving the viability of the explant. However under the proper conditions of cultivation the cells of the outgrowth may undergo differentiation and organization into complex structures resembling those of the tissue *in vivo*. Many factors influence the character of the new cells included are species differences individual variation, and tissue differences. Methods of preparation and the physical and chemical character of the medium have a marked effect on the rate and type of growth of cells in tissue culture.

There are three basic types of cell outgrowth observable in tissue cultures the discrete wandering cells of the blood and connective tissue the reticular type of connective tissue cell and the epithelial sheet or mosaic. All of these types may be present in the same culture and may temporarily assume the form of another type. Considerable study is therefore required in evaluating the composition of a culture.

### Wandering Cells

The wandering cells include granulocytes lymphocytes monocytes and macrophages. They *are the most active and they remain discrete* have a high residual growth energy and are the first to migrate from the explant this within 24 hours (figs. 228 and 230). Their source is the



Fig 228 Living 3 week tissue cultures of the same adult human bone marrow in different media. Approximately ×500 *Left* Capillaries full of blood cells and dense growth of fibroblasts in solid clot of chicken plasma and human serum *Right* Almost pure culture of macrophages and giant cells stained supravitally with neutral red in human fluid plasma containing 0 1 per cent heparin



Fig 229 Living 7-day culture of adult dog aorta Note lacy sheets of flattened fibroblasts with a few cords of spindle cells endothelium ×120

blood, bone marrow spleen lymph nodes and connective tissue. They may appear in any culture, particularly if it is of tissue which is inflamed. The granulocytes are the first to appear and are the most active. They show no polarity and travel at random by means of ruffled pseudopodia. The lymphocytes are slower to appear and are less active. They have a characteristic hand mirror shape and move in worm-like fashion by means of an apical tail. The monocytes are much larger and move very slowly by means of tremendous ruffled pseudopodia. The life of the mature granulocyte and lymphocyte is short. Immature forms survive and develop for considerable periods of time in culture. Monocytes survive for an indefinite period *in vitro*

divide and phagocytose particles of dye dead cell etc These cells do well in a medium composed simply of dilute plasma or serum at pH 7 6.

**Fibroblasts**

The reticular type of tissue is composed of cells of mesenchymal origin, the fibroblasts (figs 229 230 and 240) They either are spindle

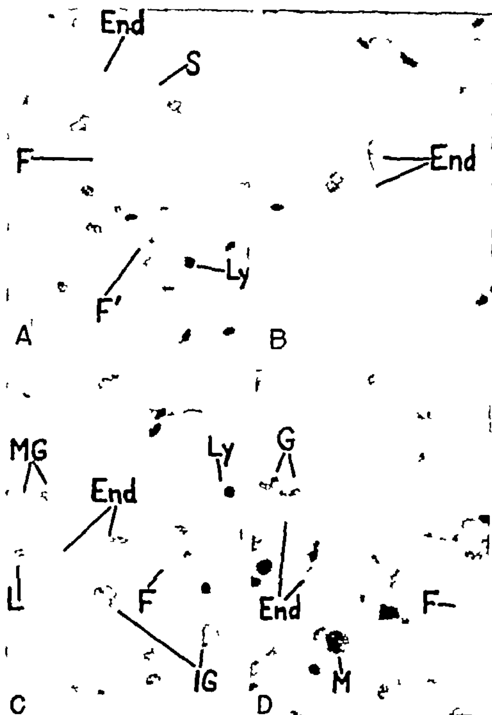



Fig 239 Stained 4 day cultures of adult chicken bone marrow Helly Mayer's hematoxylin ×730 A Capillary sprout tip (S) endothelial nucleus (End) fibroblast (F) and lymphocyte (Ly) A second capillary can be seen at bottom B Two capillary endothelial nuclei (End) C Capillary sprout containing leukocyte (L) with closely applied fibroblast (F) Note immature (IG) and mature (MG) granulocytes D Endothelial nuclei (End) granulocytes (G) and monocyte (M) (Courtesy of J F White Studies on the growth of blood vessels in vitro. I Am. J Anat., 94: 127 1954.)

cells connected loosely by long fine processes or may form a syncytium of broadly connected thin spread-out cells. They are not adherent laterally. These cells are present in most cultures, particularly in those of embryonic tissue. In many cases the cells migrate from the explant soon after the wandering cells as in cultures of bone marrow spleen, muscle, and liver. In other cultures—for example, of human skin and adult dog kidney—they grow very slowly and may not appear at all. The lag period is one to two days for fibroblasts in explants of adult tissue. The cells move by a very slow streaming of the cytoplasm accompanied by a rearrangement of cell contours and connections. Tensions within the cell may cause the appearance of fine intracellular tonofibrils. The nucleus is large and round or oval with one or several nucleoli. Fibroblasts have a low residual growth energy and in tissue culture are stimulated by the addition of various tissue extracts e.g. spleen heart kidney thyroid, pituitary or embryo to the basic medium of dilute serum or plasma. Egg white and the thrombin and globulin fractions of serum stimulate growth, which is best at pH 7.6. The presence of bicarbonate appears to be important.

## Epithelium

Epithelial growth is sheetlike (fig. 231). The cells are adherent and their surfaces are sticky. When attached they have specialized connections which do not stain like the rest of the cytoplasm and the effect is a mosaic appearance of the sheet. Sources of epithelial cells are the epidermis, the digestive, respiratory, genitourinary and nervous systems, and the glands. In rapidly growing cultures the epithelium forms a thin sheet of flat adherent cells. There may be several sheets in different planes in a thick plasma clot. In a fluid medium the cells may become isolated, spherical and remain in suspension. The physical character of the medium affects the form of the outgrowth. The rate of growth depends on the source as well as the medium. Cells from the adult epidermis, thyroid and pituitary migrate out within twenty four hours. Cells from primary explants of adult liver and kidney may not



FIG 231 Living 3½ month culture of adult dog kidney stained supravitally with neutral red. Secretory cells mainly forming channels at edge of sheet have taken up the dye in large quantity ×170

appear for a week. Epithelial cells have a large round nucleus with usually one large nucleolus. Binucleate cells and large multinucleated cells are found in cultures of liver and kidney. Cyst tubules and ducts and islands pearl and cord of cells develop in the cultures with prolonged cultivation. Under the proper conditions the cells exhibit their characteristic physiologic function.

## DIFFERENTIATION ORGANIZATION AND MODULATION *IN VITRO*

In addition to the basic cell types more highly specialized types such as bone cartilage endothelium (figs 228 and 230) all types of muscle (figs 232 and 233) and the neurons and supporting elements of the nervous system (fig. 231) have been cultivated in vitro. The growth and differentiation as well as survival of these elements are encouraged by maintaining the tissue without disturbance for as long as possible and by reducing the elements of the medium which promote rapid cell multiplication. The same conditions encourage as well as the organization and function of the connective tissue and epithelial cells. For example leukocytes have been observed to take up bacteria and foreign particles in culture. The formation of reticular collage-



Fig 232 (above) Living 16-day culture of adult human skeletal muscle. Note ribbons and round cell forms ×185 (Courtesy of Logogeff I. A., and Murray M. R. Form and behavior of adult skeletal muscle in vitro. Anat. Rec. 95: 321 1946.)

Fig 233 (below) Stained 20 day culture of adult human skeletal muscle. Cross striated ribbon Zenker PTA ×686 (Courtesy of Logogeff and Murray 1946.)

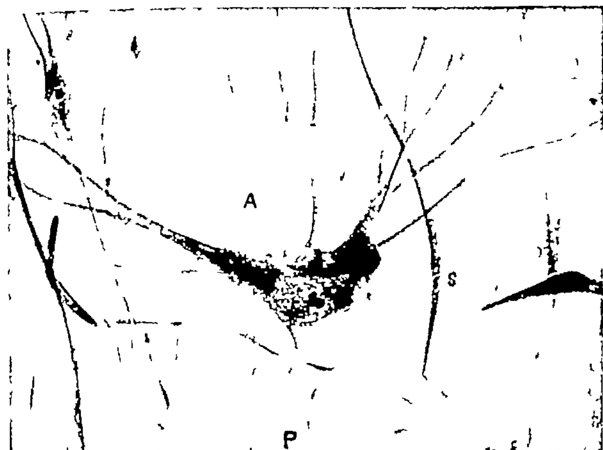



FIG. 234. Stained 24-day culture of adult human thoracic ganglion Note heavy chromidial deposit in large ganglion cell and independence of Schwann or satellite cell (S) Anterior (A) posterior (P) Zenker PTA ×234 (Courtesy of M R. Murray and A P Stout Adult human sympathetic ganglion cells cultivated in vitro. Am. J Anat., 80: 225 1947.)

nous, and elastic fibers *in vitro* has been described as well as the formation of cartilage, its calcification, and transformation into bone. The contractility of various types of muscle embryo and adult, has been maintained for months and the development of complex striations has been followed *in vitro* Epithelial tissue, both malignant and normal will form pearls, cysts, tubules and ducts and carry on its secretory functions. Thus thyroid cells will secrete colloid, kidney tubules will take up vital dyes (fig. 231) liver epithelium will synthesize glycogen, cells of the pituitary will develop specific granules, and iris epithelium will form pigment. Endothelial tubules have developed in cultures and persisted for weeks (figs. 228 and 230) Neurons from the central nervous system have been observed to differentiate morphologically *in vitro* and to develop action potentials. Changes in amount and distribution of Nissl substance have been associated with the myelinization of the nerve fiber Although the cells remain unorganized or undifferentiated in appearance they may carry on such functions as the formation of keratin by sheets of epidermal cells, the secretion of dyes by sheets of kidney epithelial cells and the contraction of isolated unstriated muscle cells.

Early workers observed that after several successive transfers, cultures of complex tissues lost their specific character This was interpreted by some workers as a "dedifferentiation" into a primitive cell type with a loss of the functional characteristics of the implanted tissue. Others thought this to be the result of the conditions of cultivation which favored an overgrowth of fibroblasts. Embryonic tissue high in connective tissue content was used largely The cultures were grown in a medium containing growth promoting substances in concentration which encouraged cell multiplication and were transferred frequently Eventually Fischer (10–14) succeeded in maintaining pure strains of epithelial cells for considerable periods of time, as well as strains of epithelial cells and fibroblasts grown in the same flask. Since that time many strains of cells have been maintained by Carrel and Ebeling (15–18) Parker (7 19) Lewis (20) Gey (21–24) Earle and others (25–29) and are on

*the market today A number of strains of human cell* are available at Microbiological Associates Baltimore Md This type of tissue cultivation has provided large amount of tissue suitable for the testing of the biologic effects of different material Cell strains have been used to advantage in the fields of cancer therapy and virology and may be eventually a source of tissue for transplantation (27 28)

Rapid cell growth with frequent subculturing results in a fairly uniform type of outgrowth. However cell strains morphologically similar have been demonstrated to retain specific physiologic differences for considerable periods under appropriate cultivation (7 10) Lewis (20) observed that successive generations of malignant cells retained their specific character through a long series of explantation and transplantation. It has also been shown that cells growing in an unorganized fashion *in vitro* resume their normal organization when transplanted back into the animal (Strangeways) (30) Similarly strains of malignant cells maintained for years outside the body have induced tumor formation when transplanted into animals (12 13 31) On the other hand permanent changes in the properties of *cell strains have occurred spontaneously (24 32, 33)* or have been induced experimentally (34–37) Cells released from the crowding tension and controls of the organism express previously suppressed potentialities or still exhibit characteristic form and function depending on the conditions of cultivation The ability of cells to assume a variety of forms and perhaps functions in tissue culture has been called modulation by Bloom (38) Weiss (39) and others. The term does not imply an irreversible change in the specific properties of the cell. Modulation is the result of the removal of restrictive conditions *which limit the activity of the cell in vivo* There is ample evidence that cells in tissue culture ordinarily retain their specific character

## GENERAL HISTORICAL REVIEW OF DEVELOPMENT OF TISSUE CULTURE

It seems appropriate to include here a brief review of the development of the tissue culture method Prior to 1907 there had been a number of reports describing the survival of organs or tissues in saline or body fluid outside the body and following transplantation to another part of the organism Experimental embryologists had observed that organs could survive outside the body for varying lengths of time in an appropriate medium At the same time the pathologists working along similar lines had developed the method of explantation in vivo" based on the idea that the most favorable environment for a transplanted organ must be within the organism itself Harrison (1) combined features of both methods He removed the tissue from the organism to a medium outside the body which approached as closely as possible the environment within the living animal. The experiment in itself was quite simple Harrison isolated a *portion of the neural tube of a frog embryo*, transferred it to a hanging drop of lymph, and observed through the microscope the outgrowth of nerve fibers from neuroblasts. None of the earlier experiments approached Harrison's in significance. This work not only proved definitely the origin of nerve fibers, a subject debated heatedly at the time but initiated the development of methods for the study of living cells from highly organized tissues isolated from the organism.

Harrison's experiment may be considered rightly as the stimulus for the rapid development *of the tissue culture method The original pro*cedure has been modified in a tremendous variety of ways to suit the problem under investigation. Various slide techniques have been used to a great extent in the field of cellular morphology where the emphasis has been on direct observation of living cells with the highest powers of the microscope These methods which consist of mounting a piece of tissue in a drop of medium on a coverslip and inverting it over a depression slide for incubation are simple and inexpensive They are ideally suited for microscopic study for short periods of time and can be followed by *staining for study as permanent preparations* Burrows (40) in 1910 devised the technique of growing chick embryo tissues in a blood plasma clot. The support given by the clot made it possible to maintain the cultures for longer periods than known previously and simplified subculture Carrel (11) introduced the addition of minced chick embryonic tissue juice to the medium as a growth stimulant in 1912. These refinements of the original technique made possible the production of large numbers of cultures of actively growing tissue From subculturing at regular intervals a variety of strains of uniformly growing cells were developed suitable

for the earliest experiments *in vitro* on the nutritional requirements of cells devised by Carrel and Baker and their associates at the Rockefeller Institute (42)

In 1911 the Lewises (43) adapted the method for the short term cultivation of tissues in a hanging drop of fluid of known chemical composition. They used saline solutions with the addition at times of bouillon dextrose or agar These media were protective rather than growth-stimulating but insured the survival of the cells for a number of days after explantation The cells grew out in very thin sheets on the glass coverslip permitting observations of living cells with the highest magnifications of the microscope. These experiments contributed a wealth of information about the characteristics of cells of blood, connective tissue epithelium muscle nerve, and malignant cells of all types (44) The Lewises were among the first to study cell activity by means of motion pictures and to correlate form with function Their use of a chemically defined medium and also the analytic studies of Carrel, Baker and Fischer were the initial steps toward the development of a satisfactory synthetic culture medium now one of the main subjects under investigation in the field of cell nutrition (42 45)

A more elaborate slide technique, known as the double coverslip method was developed by Maximow (46) in 1925 and has been used extensively since for cytologic studies and observations of cell differentiation The tissue grows on an inner small coverslip attached to the under surface of a large coverslip mounted over the depression in the slide which is usually much larger than the standard size. The cultures, being larger need less attention. The small coverslip may be slipped off for washing the culture, renewing the medium or fixation. Under conditions which do not encourage rapid multiplication the tissue can remain undisturbed for long periods of time and thus favor the differentiation of specific morphologic and physiologic characteristics of the various cell types. These slide procedures and numerous modifications of them are used extensively today in addition to the many other methods, some quite elaborate which have been developed since.

In the field of experimental embryology a method for the study of organized growth *in vitro* known as the watch glass method, was devised by Strangeways (1926) (47) and modified by Fell (1929) (48) for the study of the development of organ rudiments and the nutritional requirements of organized tissue. The tissue is grown on the surface of a plasma clot in a watch glass placed on moistened cotton inside a Petri plate. The developing rudiment is transferred every two or three days to fresh media. This method, which is suitable for quite large pieces of tissue, has been used also for the preparation of fragments of endocrine glands for transplantation by Gey (49), Martinovitch (50, 51) and Gaillard (52–55) see also (56)

The development of tissue culture along lines suitable for accurate physiologic research was promoted largely by the efforts of Carrel (57) who realized the opportunity that the method offered for study of the growth and reactions of adult tissue outside the body With Burrows he cultivated a great variety of embryonic and adult tissue by the hanging drop method but was dissatisfied with the inadequacy of these conditions for quantitative experimental work. Eventually (1923) he developed a system for maintaining tissues in a condition of uninterrupted growth in a specially designed flask. The tissues were embedded in a thick plasma substrate bathed by a supernatant fluid which could be changed as frequently as the metabolic needs of the tissue required These flasks known as Carrel flasks were large enough to permit an accurate measurement of media and concentration of test materials in it and maintenance of a constant pH. Its excellent optical properties allow microscopic observation under high magnifications. Pure strains of cells cultivated in this way have been used extensively by Carrel Ebeling Fischer Parker Earle, and others in studies of the characteristics and potentialities of various cell types and as biologic test materials.

Earle and his coworkers have developed elaborate modifications of the Carrel flask method for the maintenance of large numbers of strains of normal and malignant cells in continuous culture The use of a much larger flask accommodating ten times the wet weight of cells which covered the floor of the original Carrel flask made it possible to grow massive amounts of tissue in a much shorter time. Methods were developed for growing the cells directly on glass for transferring them in the form of cell suspensions and for measuring cell proliferation by enumeration of cell nuclei (58 59) Sanford Earle and Likely

(1948) (25) succeeded in developing a strain of cell from a single isolated fibroblast from mouse subcutaneous tissue known as the L strain. Since that time a large number of strains have been developed in the same way from other tissues. All these refinements as well as later development of other quantitative methods by Evans and others have greatly improved the accuracy of experiments employing tissue culture to determine the effect of various factors on the growth of normal and malignant cells. The use of heterologous media has produced rapid and luxuriant growth of many types of cells although there may be some question as to the application of these results (60). Evans and her associates (45) recently have contributed a formula for a synthetic medium which may replace this.

Another method of growing large amounts of tissue and used by many workers since for maintenance of cell strains is the roller tube technique developed by Gey (61) in 1933. The cultures are planted in thin-walled test tubes which are placed in the incubator in a rack equipped with a motor that keeps the rack in constant slow rotation. By this means the tissue is washed by the fluid medium. The cultures may be placed directly on the glass wall of the tube or in a plasma coagulum. This method has proved to be an ideal and simple way of keeping cells in continuous culture with a minimum of attention. More recently workers in the field of virology have found that the maintenance of at least some strains does not require constant rotation and have left their cultures stationary (62-64). Porter (65) has modified the tube by blowing the rounded end into a Carrel flask for better observation. Pomerat (66) has combined features of the slide and roller tube by growing tissues on coverslips placed in roller tubes.

Tissue fragments have been cultured in suspension for many years. Maitland and Maitland (1928) (67) originally used suspensions of kidney tissue in dilute serum for the cultivation of vaccinia virus. Some years later fragments of adult tissue were grown for short periods in small Erlenmeyer flasks by Parker (1936) (68) who in studies of antibody formation desired to remove the fluid for titration of antibodies. In these studies the condition of the tissue was of great interest. In early work along these lines virologists were interested chiefly in promoting the multiplication of virus. Simms (69) in 1942 devised a method for the propagation of virus in tissue grown also in an Erlenmeyer flask in dilute ox serum ultrafiltrate, a medium free of protein. This procedure was successful and widely used for a time. Later, however, the roller tube technique was used extensively until in 1948 and 1949 Enders, Weller and Robbins (70, 71) succeeded in growing poliomyelitis virus in cultures of suspended cells from various human extraneural tissues. Gey, Syverton and Scherer (23, 63) found that a stable strain of malignant epithelial cells proved to be highly susceptible to poliomyelitis and has provided a prolific and easily managed source of suspended cell cultures for the growth assay and study of virus and host-virus relationships. These cells are grown in tubes or bottles in dilute serum and rotation of the vessels has not proved to be necessary. In 1922 Carrel (72) made a study of normal and diseased leukocytes grown in a fluid medium, dilute serum, in Carrel flasks. Since that time Osgood (73) has developed methods for growing the leukocytes from normal and leukemic human beings in large bottles with a constant flow of fluid media and gaseous atmosphere. As is evident, a great variety of vessels has been used successfully for the cultivation of tissue outside the body—Petri plates, tubes, bottles and flasks of all sorts. The trend has been toward simplification of methods and greater accuracy.

## RECENT TECHNICAL DEVELOPMENTS AND THEIR APPLICATIONS

Cells isolated in tissue culture are ideal subjects for the study of the fine structure of protoplasm. The increasingly refined methods of observation available today make possible correlation of the physiologic activity of the cell with its intracellular organization. During the past half century cinemicrophotography has been used to record the characteristic movements of different types of normal and malignant cells and to correlate cell form with function (74-78). The adaptation of phase microscopy to tissue culture within the past ten years has made it possible to see far greater cell detail than is observable with the light microscope. A comparison of the structure of a given cell before and after fixation by this method can help to determine what features of the fixed cell are actual and what are artifact.

The combination of phase optics and time lapse photomicrography initiated by Gey (79) has added to the possibilities of observing directly the activities of living cell (fig. 23b). Gey (80)

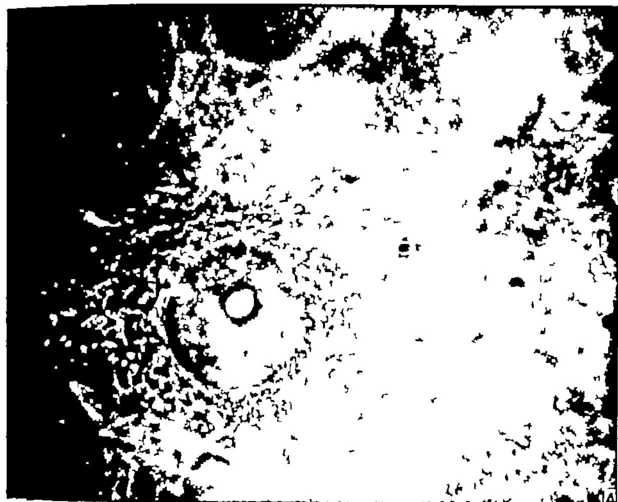



FIG 235 A. Rat normal fibroblast strain 14 p

has compared the structure of cells in the living state recorded by this method with that of the same or similar cells fixed and studied by the electron microscope. The activities of cellular substructures have been observed in minute detail by this method. Hughes (81) has studied mitotic activity of cells in culture by a combination of cinephase and polarization microscopy. Observations of the movements of cell nuclei, nucleoli, and mitochondria have been made with cinephase microscopy at high magnifications by Chèvremont and Frederic (82), Lettré (83), Pomerat (84), and others, and the direct response of these organoids to various experimental conditions has been recorded.

Development of methods for providing cultures with a constantly renewed nutrient medium was initiated by Burrows (85) many years ago. Later deHaan (86) and Carrel devised more elaborate methods for the circulation of a large volume of fluid. More recently Osgood (73) has designed a system for large scale culture in constantly renewed fluid media of cells from the blood and bone marrow of normal and leukemic human beings. One of the most satisfactory of these perfusion chambers, developed by Buchsbaum (1954) (87) for observation of the direct action of drugs on living cells, permits automatic continuous flow of the medium at known rates under known gaseous tension past the cell under observation (fig 236). The medium can be changed without interruption of observation to allow study of the effect of different concentrations and degree of injury and ability to recover in normal medium. By the use of cinephase microphotography the responses of living cells to a variety of chemicals have been studied, and the method should prove most valuable for further pharmacologic studies.

Cells grown in tissue culture have proved to be useful for the investigation of submicroscopic structures with the electron microscope (65, 88). The power of resolution of this instrument many times greater than that of the light microscope makes possible the direct study of fixed protoplasm in detail. The advantage of the



FIG 235 B Tumorous strain T-333 derived *in vitro* from 14 p cell are larger thicker and contain a conspicuous ring of inclusion droplets Living images with bright phase contrast ×1500 (Courtesy of G O Gey P Shapras and F Borysko Activities and responses of living cells and their components as recorded by cinephase microscopy and electron microscopy Ann New York Acad Sc 58:1089 1954)

tissue culture as a specimen for study is its very thinness which permits scrutiny of an intact cell, with its composite parts in normal relationship Whole cells as they occur *in vivo* can be examined only in thin section It is not possible to study living cells by this method. The ultraviolet microscope which has a resolving power several times greater than that of the light microscope has been used to advantage also for the photographic study of details of fixed unstained cell The recent development of the Land ultraviolet color translation microscope offers a new opportunity for the study of the fine structure of cells

When ultraviolet radiation is used to excite fluorescence such phenomena can be observed directly in living tissue cultures The administration of fluorescent dyes permits observations of the chemical nature and affinities of the various parts of the living cell. Observations of the activities of the cells in tissue culture following such treatment indicate the effect of the compound on cell vitality (89) The use of tissue cultures in cell physiology using radioactive isotopes has many advantages (90–92) The incorporation of tracers in the medium takes place during the continuous cultivation of the cells under controlled conditions and observation of the effect on the cells can be made simultaneously The uptake of an isotope can be measured by the Geiger Müller counter or other means and localized by radioautography Under favorable circumstances it is possible to locate these elements intracellularly This method holds promise for the study of the biosynthesis of labeled organic compounds

The technique of micromanipulation of cells and tissues under high magnification developed by Chambers (93) in the 1920s has added greatly to the knowledge of the physical and

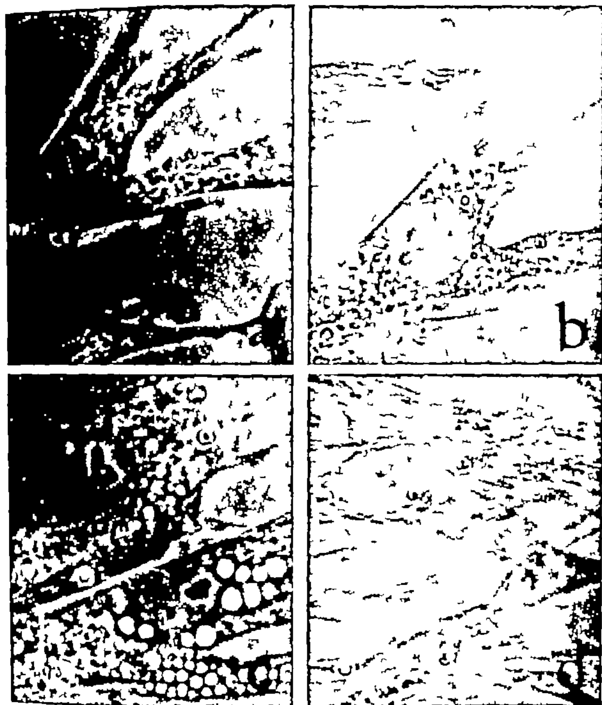



Fig 286 Action of morphine sulfate (3.3 mg per ml) on chick embryo fibroblasts in a perfusion chamber Phase contrast photomicrography nuclei about 10 microns in diameter a Normal cell b After treatment formation of vacuoles c Cell with large vacuoles which continue to form long after drug is washed away d Five hours after beginning of perfusion cells are essentially normal (Courtesy of Buchsbaum R. and Kuntz J A. (87))

chemical properties of protoplasm. In Chambers own words (1050) cytoplasm can be punctured with impunity filamentous mitochondria can be cut in two and vacuolar membranes ruptured. On the other hand, the nucleus when ever so slightly punctured, undergoes death changes. Furthermore the irritability of myoblasts can be tested by prodding. Operations on sheets of cells in culture can be followed by studies of the regenerative capacities and activities of different tissues. It is possible with the tissue culture method to conduct such experiments with isolated living vertebrate cells, and it is to be hoped that eventually this will be possible with single human cells Physiologic studies of the properties of such specialized tissues as muscle and nerve

have been reported by Chambers, de Renyi and Hogue (94), Peterfi (95) and Levi (96). In 1933 Peterfi and Williams (95) studied the response of cultured nerve cells to electric stimulation. At about the same time Goss (97) reported a method for recording potential differences in cultures of heart muscle from rat embryos by microelectrodes moved about by a micromanipulator. Recently Crain (1950) (98) has obtained intracellular as well as extracellular potential patterns in cultures of chick embryo spinal ganglia while under high power microscopic observation.

Bush, Duryee and Hastings (99) have developed a new model electric manipulator to dissect out the nuclear and cytoplasmic organoids of single living frog cells (fig. 237). Duryee has studied isolated nuclei, nucleoli, and chromosomes and compared the organization of somatic nuclei with that of germ cell nuclei previously studied exclusively in isolated eggs. By this method it has been possible to treat the nuclear membrane chemically to determine its constitution. The extrusion of nucleolar and nuclear material into the cytoplasm has been observed and interpreted as evidence of the secretory activity of the nucleus important to the protein metabolism of the cell.

The very sensitive micromethods for measurement of enzyme activity developed by Linderström-Lang and his coworkers at the Carlsberg Laboratory have been applied to the study of the respiration of cells grown in tissue culture (100). The development of increasingly refined micromethods such as the Cartesian diver and its modifications hold great promise for the correlation of the biochemistry of the single mammalian cell with its integral parts. Micropipettes, a feature of Chambers's original microdissection apparatus, used by him to inject dyes, chemicals and other materials, have been adapted since by cytochemists for the application of chemical agents to individual cells and even to localized areas within a given cell. This technique can be utilized for the analysis of chemical reactions in microdrops of material withdrawn from a single cell. Kopac (1953) (101) has improved the Cartesian diver method



Fig. 237. High magnification of a single frog kidney adenocarcinoma cell undergoing microdissection with three needles. Middle one in nucleolus. Phase contrast. (Courtesy of Duryee, W. R. and Doherty, J. K.: Nuclear and cytoplasmic organoids in the living cell. Ann. New York Acad. Sc. 58: 1210, 1954.)

so that much smaller density differences can be measured with smaller reaction volumes. By means of a submicromanipulator samples of cytoplasm from 0.3 to 10 micro-microliters have been removed from different areas of single living vertebrate cells stratified by ultracentrifugation and analyzed for enzyme activity. It is possible to localize enzymes in the cell by this method—in other words, to correlate specific chemical activity with definite particulates or areas of the cell, and thus to decide, as Theorell (1956) (102) has said recently "how the enzymes are arranged in the cell structures. This implies, as a matter of fact, the filling of the yawning gulf between biochemistry and morphology."

## RECENT TRENDS IN TISSUE CULTURE RESEARCH

### Cell Nutrition, Metabolism Growth and Development

The development of new techniques has made possible more exacting studies of cell growth and activity. The present emphasis is on investigation of the causes of human pathologic conditions with the aim of controlling disease. The morphologic characteristics, specific activities, methods of locomotion, multiplication rate of growth ability to regenerate, and gross nutritional requirements of a variety of tissues, many human have been described. Epithelial, mesenchymal and more specialized tissues have been found to have different growth characteristics. Tissues of the same type vary according to origin—species, individual and tissue (19 103 104). The character of the outgrowth in tissue culture can be influenced by changing the conditions of cultivation and is affected particularly by the composition of the medium.

By means of refined studies of the effects of agents on the cytology and physiology of single cells and tissues an understanding of abnormal growth may be gained. It is necessary first to determine the characteristics of normal growth and this can be undertaken to advantage at a cellular level by tissue culture under carefully controlled conditions. Waymouth (1954) (104A) has written an excellent review of the contributions of tissue culture to knowledge of cell nutrition. (Also 104B.) For such studies the ideal circumstances require the use of cells of common origin in a medium of known composition. The study of cell metabolism *in vitro* has been carried on largely with established strains of cells on the assumption that these strains consist of stable homogeneous cells. Many cell strains established *in vitro* have shown such stability for years [Carrel (1914) (3) Ebeling (1922) (14) Fischer (1939) (31) Gey (1936 1954) (21 24)]. However many workers do not recommend maintenance of a strain for experimental use for more than a few months. Others prefer to use freshly explanted tissues or primary stock explants for their experiments (66 104B 104C 104D). The stable strains of malignant cells carried by Lewis (20) were frequently passed through animals. Perhaps because of rapid cultivation or use of heterologous media some strains of cells have developed malignant properties spontaneously or have lost malignant characteristics or shown other variations from the original (32, 33 36 37). Even strains derived from a single cell may show marked deviations in appearance and physiologic properties due to spontaneously or experimentally produced changes, perhaps "mutations" (105 106).

Many recent reports of altered nutritional requirements of human cells in continuous culture [Chang (1957) (107) Puck (1957) (108)] and the development of malignant properties by strains of normal human epithelial cells [Moore (1957) (109)] would indicate need for a careful re-evaluation of the methods of cultivation and interpretation of results. Parker in 1957 (106) pointed out that strains of "altered cells" have been developed that cannot readily be identified with any cell type existing in the animal body. They are characterized by their rapid multiplication to form uniform populations of free living cells thriving under conditions unfavorable to the cells from which they were derived. Such cells may change in their relation to viruses and as indicated above, are potentially malignant. Therefore until more is known about the causes of changes in cell strains strict criteria for stability should be established before cells under continuous cultivation are used for replacement.

At the present time there are a number of chemically defined nutrient media capable of maintaining cell strains for prolonged periods of time (45 110). For reviews of the development of synthetic media see the accounts by Morgan (42) Waymouth (104A) and White (9). None of these media is adequate as yet for the differentiation *in vitro* of highly specialized tissues. In most work with cell strains there has been an emphasis

on rapid multiplication and production of massive amounts of rapidly dividing cells of uniform appearance. It is equally important to the studies of cell physiology and experimental pathology to maintain cells in a stable condition as they are in nature, to encourage differentiation of specialized cells, and to use such cultures for the study of factors which influence the normal function of highly organized tissues. The organ culture methods developed by Strangeways and Fell (1926) (47) have been applied recently to studies of the influence of vitamins and hormones on growth and development (111). The same methods have been used to study the relations of cell types and the influence of one cell on another during growth and differentiation. Experiments with tissues in early embryonic stages by in vitro methods by Waddington (1937) (112) and Holtfreter (1948) (113) have contributed to the knowledge of the developmental potentialities of vertebrate tissues. Dissociated organ rudiments and component cells have been combined for the study of organizational influences. Moscona (1952) (114) has combined chick embryo cells dissociated by enzyme action, and Grobstein later (1953) (115) isolated rudiments of the mouse, and their works have shown that the morphogenesis of certain epithelial structures is dependent on the presence of associated mesenchyme. Moscona (1956) (116) has reported such organizational activities of cells going on in suspension in a fluid medium. Grobstein (1956) (117) has cultivated tissues on either side of a millipore filter to distinguish between inductive effects due to cell contact and those due to diffusion, and he concluded that the intercellular ground substance may play a part. In this connection it should be noted that the digestive action of a variety of enzymes is being used to facilitate dissociation and handling of tissues without at present a correlated study of the specific effects of the enzyme involved. Trypsin, for example, has been found to stimulate growth of mesenchymal elements in concentrations which inhibited epithelial outgrowth (101D).

## Pharmacology

Cells isolated in tissue culture provide a particularly sensitive means of testing the effect of biologic, chemical and physical agents on cellular appearance, growth and metabolism. Different types of cultures have been exposed to the action of vitamins, hormones and enzymes, tissue extracts and protein fractions presumably thought to stimulate growth, drugs of all kinds, respiratory and mitotic poisons and carcinogenic compounds. Carrel and Ebeling (1922) (?) introduced the use of leukocytes from adult human blood for nutritional experiments and studies of the properties of the cells and sera in which they were cultivated. Carrel (1934) (118) observed that the morphologic character of the leukocytes was modified by the serum medium. In other words normal cells cultivated in pathologic serum took on the appearance of the cells of the diseased individual in autologous serum and vice versa. The rates of migration and phagocytic activity of leukocytes in tissue culture were used during World War II to evaluate the toxic effects of antibacterial agents (119-121).

In the author's laboratory studies were made of the growth response of adult connective tissue fibroblasts and skin epithelial cells to several hundred substances of possible use in the treatment of wounds (101D). These included bases, antibacterial growth stimulants such as the constituents of the blood, tissue extracts, proteins and protein digests, vitamins, enzymes, hormones and other organic and inorganic compounds. In most instances substances reported clinically to stimulate wound healing were found to be inert or toxic (e.g., chlorophyll, cod liver oil fractions, glycerine, pectin, triethanolamine and urea compounds). Many commonly used bactericidal substances such as boric acid, hydrogen peroxide, iodine, phenol, Zephiran and zinc peroxide in very low concentrations inhibited growth. A large number of miscellaneous organic compounds recommended for antisepsis, for example the acridine antiseptics, were found to be highly toxic. The sulfa drugs were well tolerated as were purified preparations of penicillin and streptomycin. This was true also of the more recently developed "mycin" drugs. On a weight basis penicillin preparations have a markedly low level of toxicity in vitro compared to other antibiotics.

The effect of all these drugs on growth in vitro varied from toxic to inert to growth-stimulating according to both the concentration and the tissue used. Similar results have been observed following prolonged treatment of tissues with cold or with freezing and it has been suggested that just sufficient injury may occur to cause some autolysis with the production of

growth stimulants The possibilities to be considered from such a wide range of reaction are important in view of the general and sometimes prolonged use of antibacterials. The nature of the action of these substances will be understood only when more is known of the metabolism of normal and abnormal growth. The variation in response of different tissues to agents such as trypsin, thyroid extracts and ascorbic acid has been observed. In general epithelial tissues are more sensitive than connective tissue. The tissue culture technique provides an excellent means of comparison of the effect of chemicals on different types of tissue and of observing the direct effect of the agent on a specific tissue at a cellular level

More recently Pomerat and his coworkers (1954) (122) have reported studies of toxicity reactions of cultures of embryonic chick heart, spleen, and spinal cord and of adult human skin epithelium to a variety of drugs of interest clinically These authors observed a high toxicity of antihistamines for skin epithelium and believed that this action may be related to the "sensitivity" which can follow the topical use of antibacterial ointments. Alkaloids were mainly toxic to tissue culture growth Comparison of animal studies with tissue culture results indicated that the intact organism is more sensitive to certain compounds acting on the nervous system than isolated cultures of chick spinal cord. These experimenters have cultivated tissues from adult human brain and suggest the use of such cultures for physiologic and pharmacologic studies.

Verne (1954) (123) has reported differences in sensitivity to metallic salts shown by cultures of nervous tissue epithelial cells and fibroblasts, and also variation in response of strains of fibroblasts from different organs. The sensitivity of cultured cells differs from that of the whole organism and may be greater or less. Cells *in vitro* may acquire resistance to toxic drugs, which persists, however for only a few cell divisions after the drug is withdrawn.

Another group of workers mainly European have used the organ culture method to study the effect of hormones and vitamins on isolated developing tissues Thus Gaillard (1942) (52) and later Wolff (1952) (124) have shown the direct influence of sex hormones on developing gonads. Hardy and her associates (1953) (125) have reported the cornification of vaginal epithelium *in vitro* in the presence of estrogens. The direct toxic action of cortisone on lymphocytes in lymph nodes grown *in vitro* was reported by Trowell (1953) (126) A group under Fell (1954) (111) observed that L-thyroxine accelerates the differentiation of chick skeletal tissue *in vitro* initially stimulating growth and later retarding it. Different osseous tissues showed varying patterns of behavior Insulin on the other hand, retarded differentiation. Pituitary growth hormone had no effect on similar cultures indicating an indirect effect *in vivo* Vitamin A had a severe direct effect on skeletal rudiments, causing dissolution (127) This vitamin also inhibited keratinization of chick ectoderm (128)

The reaction of chick embryo fibroblasts to a number of drugs, stimulants and depressants, was observed by Buchsbaum (1954) (87) by means of the perfusion chamber *mentioned* above This method allows observation of cell recovery following removal of the drug and also study of the effect of repeated small doses as opposed to one large one. Several different patterns of response were observed, all reversible with the concentrations used Pentobarbital sodium Pentothal sodium phenobarbital barbituric acid, and adrenalin chloride caused withdrawal of processes and formation of *blebs* at the cell surface. Codeine, Benzedrine and morphine sulfates, and strychnine sulfate in low concentration produced an accumulation of vacuoles in the cytoplasm (fig 236) The *degree* of response was influenced by concentration and time Response to repeated small doses differed from that to one large dose In higher concentration strychnine sulfate caused a violent *boiling* of the cell

Such changes in surface phenomena as well as in the reactions of other specific parts of the cell to different compounds as a result of the direct action of chemical agents are a source of information about cell chemistry and metabolism. The activity of mitochondria in chick embryo fibroblasts has been observed and recorded by Chèvremont and Frederic (1952) (82) who observed structural changes and eventual destruction caused by detergents considered to block metabolic exchanges at the cytoplasmic interface Substances known to affect cellular metabolism and enzyme activity such as 2 4 [illegible] and phenylurethane modified [illegible] form of the mitochondria, nucleus [illegible] These activities are considered [illegible]

of metabolic exchanges between mitochondria and cytoplasm.

### Cancer Research and Diagnosis

The effect of drugs on mitosis has been studied widely by the tissue culture method since Ludford (1939) (129) observed the direct action of colchicine and the so-called mitotic poisons on cells in vitro. In the search for a means of controlling the growth of tumors these substances as well as those which inhibit or augment the effect of the poisons have been investigated to learn the chemistry of cell metabolism, division, and growth (130).

The Lettrés have analyzed the chemical effects of a number of substances on the metabolism of chick mesenchymal fibroblasts. They have studied the action of drugs which are respiratory and mitotic poisons and their synergists and antagonists. Cultures treated with substances altering cell respiration produce different effects. Cell division almost stops in the presence of pyocyanine, which increases oxygen uptake. Under anaerobic conditions the cell surfaces become very active. Differential responses to anaerobic conditions on the part of normal and malignant cells have been reported. Lettré has investigated the relation between various chemicals important to cell metabolism and the activity of mitotic poisons. The action of acriflavine, which causes clumping of chromosomes by combining with nucleic acid, can be counteracted by the addition of nucleic acids. The organometallic poisons are antagonized by addition of SH compounds. Colchicine, a spindle poison, is considered by Lettré to act by inhibiting a reaction between adenosinetriphosphate (ATP) and the contractile system of the spindle. It was observed in Murray's laboratory that meso-inositol and tropolone reversed the colchicine effect (131, 132). The blockage of mitotic inhibition caused by the fluorophenylalanines by L-phenylalanine was reported by Biesele and Jacques (133). These authors consider colchicine action antimetabolic in a chain of reactions necessary for nuclear division. There is considerable argument concerning the means of action of mitotic poisons. The fact that agents widely different chemically are effective in counteracting the effect of these poisons indicates that the process of mitosis is extremely complex.

Analyses of the effect of chemical agents on the cell and its parts is yielding information on normal and abnormal cell metabolism. Colchicine experiments have led to a search for chemicals with a selective deleterious effect on cancer cells. It is important, as indicated above, to see how and on what part of the cell an agent acts, and for such research tissue culture is an ideal tool. There are a number of types of materials observed to inhibit differentially the growth in vitro of malignant cells from normal cells. Heptanal sodium bisulfite was found to inhibit selectively the growth of human mammary tumor cells (134). Cornman (1944) (135) reported the toxic action of crude penicillin for rat sarcoma cells and selectively damaging effects on other animal tumors of podophyllin (136). Nitrogen mustard (137), alkaloids and acridines (138), Myleran (139) and other chemicals have been reported. Unfortunately, although there have been reports of correlation of in vivo with in vitro results (140), there also have been discrepancies and a consequent reluctance to accept the tissue culture method as a valid approach to chemotherapy (141). For example Biesele (1954) (142), who has compared the relative toxicity for normal mouse tissue and mouse sarcoma 180 of a large number of chemicals, including 2,6-diaminopurine, 6-mercaptopurine, and a number of folic acid analogues, reported inconsistencies in in vitro and in vivo results and species variation. Murray and her coworkers (1954) (143) have, for this reason, recommended testing agents directly on cultures of human materials. They have reported selective inhibition in vitro of human glioblastoma by 8-azaguanine and Myleran. The wide individual variation in tumor growth, variability in growth and division rate in different areas of the same tumor, as well as species differences and discrepancies between tissue culture and clinical results observed by these experimenters have led them to stress the importance of screening agents directly on cultures of the human tumor to be treated.

However, it is generally conceded that progress in chemotherapy depends on the search for a chemical factor lacking in the cancer cell (144). The fact that different tumors show a differential reaction to chemical agents, as pointed out above, and a different reaction in vitro in different media indicates that the metabolic difference between a normal and a malignant cell is not a simple one and not necessarily a constant one. From this one point of view the tissue culture method can and is proving to be

invaluable in investigating the action of antimetabolites on the cell and the mechanics of nucleic acid synthesis. By microscopic observation the effects of chemical action on the living cell can be observed directly and the manner of action determined. Tissue culture can be of far greater value as a means of studying mode of action, metabolites and antimetabolites and differential effect on cells than as a screening device. Studies which show the completely different character of cell strains developed from the offspring of a single cell (145) indicate the potentialities of adaptation of normal and malignant cells which may be responsible for the resistance to carcinolytic drugs eventually acquired by treated tumors. Tissue culture methods should prove to be of great value in the analysis and control of this phenomenon by providing a better understanding of cell metabolism. The use of viruses as chemotherapeutic agents has been discussed (146) and the possibilities of studying the host-virus relationship in tissue culture from this point of view are of current interest. Also current is the idea of attenuating an effective anticancer virus in tissue culture for clinical use.

The use of tissue cultures of human tumors for histogenetic studies has proved to be helpful in the differential diagnosis of tumors particularly those of the nervous system. In the unrestricted environment of the culture tumor cells assume a characteristic and distinguishable form not evident in sections. In 1928 Kredel (147) introduced this method for the study of a series of intracranial tumors. Russell and Bland (1933) (148) later described the behavior of a variety of human neurogenous tumors *in vitro* and with Canti, made motion pictures of these cells. Murray and Stout (1954) (149) in a review of the subject, confirm from their own observations the individuality *in vitro* of the astroblasts of glioblastoma multiforme sufficient for a ready diagnosis. These authors (1940) (150) and Chlopin (1943) (151) independently determined by a comparison of the growth in tissue culture of the neurilemoma, or nerve sheath tumor with that of normal peripheral nerve that this tumor was derived from the Schwann cells. Murray and Stout (1942) (152) have established also the origin of a group of previously undiagnosed vascular tumors, the hemangiopericytomas, neoplastic growths of capillary pericytes. In 1947 they (153) used tissue culture successfully for the rapid and reliable diagnosis of the sympathicoblastoma. Tissue cultures have been useful in the detection of Hodgkin's disease and in the study of its characteristics (154–155). Tissue culture studies of these and other neoplasms have been of value not only in the diagnosis of tumors but as a means of observation of form and activity of living malignant cells (156–160).

### Radiobiology

Investigation by means of tissue culture of the problems associated with radiation treatment of malignant disease has been of the greatest importance. By this method it has been possible to study and observe directly the effect of irradiation on living normal and malignant cells. From the earliest radiation studies it has been known that embryonic and undifferentiated tissues are most sensitive, that cell division is disturbed, and that the chromatin is the most sensitive part of the cell. In 1914 Price-Jones and Mottram (161) observed the inhibitory effect of radium rays on the mitosis of carcinoma cells *in vitro* while cell migration was unaffected. The changes which occur in irradiated tissue were observed directly and recorded in motion pictures by Canti (1928) (76). These changes are the result of the direct action of the radiation on the cells. Strangeways (1924) (30) and a group at his laboratory in Cambridge since have carried on an extensive series of experiments on the effect of radiation on mitosis and the cell, and the factors which modify radiation effects (162–164). These studies have indicated that different cells respond differently to the same quantity of irradiation. There are fundamental differences in reaction according to time and intensity. The biologic effect increases with an increase in intensity up to a critical value. Ludford (1932) (165) recorded early changes in the non-dividing nucleus and Lasnitzki (1943) (166) has shown that sufficiently high doses of irradiation lead to the breakdown of cells in the resting period. These fundamentals have influenced the clinical usage of radiation in the treatment of cancer. Resistance to radiation which can develop in tumors over a period of treatment has also been demonstrated in irradiated tissue cultures which provide an opportunity for the study of this effect. Strains of cells from sections of human tumors cultured following irradiation may have characteristics quite different from those established from an earlier biopsy (24).

Recently Bloom Zirkle and Uretz (1953, 1955) (167 168) developed methods for the selective irradiation of small areas of individual cells with microbeams of protons and ultraviolet light They have recorded with cinemicrophotography the aberrations of irradiated chromosomes the inhibition of different phases of mitosis and the specific effects of different types of rays on the cytoplasm which result in marked abnormalities of cell division. Experiments of this kind are valuable not only for an understanding of the mechanism of radiation injury but also as a source of information on the normal action of different cell parts.

## Tissue Transplantation

The possible applications of culture technique to the field of tissue transplantation are many Tissues have been maintained or conditioned *in vitro* prior to their use as grafts. The cultivation of tissue *in vitro* provides a simple and sensitive means of evaluating the effect of different methods of storage on viability Although this method provides an excellent approach to the study of the effect of implantation on the grafted tissue and an analysis of the host-graft relationship tissue culture has not been used to any extent for this purpose. Most workers have drawn conclusions as to the condition of grafts before and after implantation, from their successful take or function and from histologic studies of the tissue usually following removal of the graft from the host There is no doubt that the maintenance of fragments of tissue outside the body by culture is possible and may be of great clinical importance particularly in skin grafting and in the treatment of glandular deficiencies Parker (1936) (108A) demonstrated by culture methods that small pieces of tissue could be stored for a year or more in serum in a viable state The importance to the surgeon of a ready source of suitable viable material for grafting should encourage the use of such methods

It is debatable whether the cultivation of the tissue in the recipient's plasma actually conditions the tissue for homografting or is the factor essential for the success of the graft In 1931 Stone Owings and Gey (49) reported the results of a long series of experiments with dogs and some humans who had received homologous and heterologous grafts of thyroid and parathyroid tissue Small fragments of these glands had been grown in the recipients plasma and serum for two or more weeks before implantation. About 50 per cent of the homologous thyroid grafts in dogs were successful Two humans with parathyroid deficiency had been treated successfully with cultures of human material (age of donor not specified) The authors considered that the tissue culture treatment may have contributed to the success of the grafts but stressed the importance of other factors such as the site of implantation, the very small size of the graft—at times a thin sheet of living cells—and the choice of material from very young individuals. Gey recommended the use of tissue from the newborn when possible Lux Higgins and Mann (1937) (169) carried out experiments along similar lines implanting tissue cultures of adrenals from newborn rats into deficient adult Fifty per cent of their grafts were successful and there was evidence of the survival of part of the grafted tissue as a functioning gland. However only half of the grafts conditioned by culture in the recipients serum survived and this was not considered sufficient evidence of an *in vitro* modification of biologic differences The size of the graft the age of the donor and the genetic relationship were considered to be the important factors

Studies by Landsteiner and Parker (1940) (170) in relation to antibody formation have indicated that chick fibroblasts grown for nearly 8 months in rabbit media retained species specificity as shown by positive precipitation reactions of the tissue culture fluid with immune sera from rabbits injected with chicken serum More recently Gaillard (1948 1954) (53 54) has used the culture method to adapt small fragments of parathyroid tissue from newborn infants in media from the recipient for grafting in human cases of postoperative tetany In a series of 30 patients there were 7 with long term positive result All of these were patients under 32 years of age Gaillard has reported also the successful grafting of cultures of fetal ovary in humans who gave some evidence of subsequent hormone function (1950) (55) Conway and his coworkers (1953) (171) also have reported that the survival of homografts of the skin of mice was prolonged by cultivation *in vitro* from one to 9 weeks before grafting Martinovitch (1950 1952) (56 1) successfully transplanted into the anterior chamber of the eye of adult rats a variety of endocrine organs of infantile rat organs such as the adrenal pituitary thyroid parathyroid, pan-

creas, and pineal gland following cultivation *in vitro* for months. In certain instances small fragments of homologous tissue have been transplanted successfully. Whether this success has been due to previous treatment of the graft, to size of graft, site of implantation, age of donor or recipient, genetic relationship, or a combination of factors remains to be established.

Since the time of Carrel the method of tissue culture has been used for the propagation of cell strains which have served as a continuous source of living cells for experimental purposes. Recently Earle and his coworkers (26–28) in collaboration with the Tissue Bank of the National Naval Medical Center have carried out long term cultures of human skin with the object of developing a pure strain of epidermal cells for the study of the behavior of normal human skin epithelium and as a possible source of cells for clinical use. An initial strain of mixed epithelial cells and fibroblasts eventually assumed the morphologic characteristics of the latter type. Strains of cells from the epidermis separated from the dermis by the action of trypsin also showed a change in form. However similar strains isolated from mouse skin were observed to resume their differentiated state when transplanted to the anterior chamber of the eye, or autografted to denuded areas isolated by transparent observation chambers. These authors (1956) (28) have now succeeded in establishing from the skin of a 65-year-old man a pure strain of epithelium which is more than three years old and which can be handled as a suspension to produce massive quantities of cells. Epithelial cells from a substrain produced by single cell isolation, when injected into the hamster cheek pouch to determine neoplastic tendencies, produced growths *in vivo* over a period of nine months. These were judged to be non malignant (172). Further studies are in progress to evaluate the clinical usefulness of such strains.

Although as early as 1912 Carrel (173) recommended the cultivation *in vitro* of preserved tissue as a test of its viability, he did not culture the actual grafts but drew conclusions as to their state of preservation from cultures of embryonic tissue stored in the same way. Since then conclusions regarding the advantages of different methods of storage have been drawn largely from the macroscopic and microscopic appearance of the tissue and from transplantation results. These are not reliable evidence of the state of viability of the tissue at transplantation. The method of tissue culture, on the other hand, provides "a crucial test of viability" (174) (cf Weiss and Taylor 1940) which makes possible an evaluation of the effect on the same piece of tissue under a variety of experimental conditions. Within the last five or ten years there has been a renewed interest in the use of the tissue culture method for the study of optimal conditions for the survival of large pieces of tissue for replacement purposes—in particular skin and blood vessels. The results have indicated that viability of tissues can be maintained for much longer periods by methods other than those in use ten years ago.

Factors which determine the ability of a tissue to survive after removal from the living animal are source, size, method, medium, temperature and length of storage. Every study of tissue survival indicates that there is tremendous variation in the ability of the tissues of different individuals to withstand adverse conditions. The different tissues of a given individual vary also in their ability to survive (175–177). The human epidermis appears to survive far better under adverse treatment than does the aorta. Tissues with a low water content appear best able to survive low temperatures. There are also species differences. For example, human red cells and spermatozoa survive storage at subzero temperatures better than similar cells of other species (178).

Size is an important factor. While small fragments of tissue have been shown to survive months and perhaps years in a maintenance medium at physiologic temperatures (168A), by current methods of preservation large sections of tissue or organs have not remained viable for more than a month or two, even at temperatures just above zero, where metabolism is greatly reduced (179–181). Single cells or extremely thin slices of tissue apparently withstand freezing better than large pieces. Small fragments of tissue are particularly sensitive to temperatures slightly below zero, while large pieces of tissue do not survive long at high temperatures.

The importance of immediate refrigeration of tissue after removal from or death of the animal has been stressed many times. The author and coworkers observed that a certain number of cells of adult dog aorta, left *in situ* following the death of the animal, survive for as long as ten days if the dog is refrigerated immediately, while cells remain viable for only five days when the dog is left at room temperature for several hours before refrigeration (182). Similarly sections of large

human arteries taken many hours postmortem and stored for several days in saline at icebox temperatures fail to grow in tissue cultures (104)

For many years tissues have been stored for use as grafts in moist air Ringer's Tyrode's or other physiologic solutions, or in saline sponges or Vaseline gauze at icebox temperatures for short periods up to five or six weeks. Comparative grafting experiments correlated with tissue culture tests have indicated that viability can be maintained for much longer periods by storing the tissue in a nutrient medium, at least at the temperatures tested. This is not surprising since such a medium provides nutritive materials and oxygen, buffers the acids and dilutes toxic material

The importance of viability as a factor determining the success or failure of grafts of large pieces of organized tissue has been much discussed. Freshly grafted tissue is not maintained in the host in an unchanged state The percentage of the living elements of the autograft which survive is not known but several studies indicate that viability of fresh autografts is reduced greatly following implantation (183 184) The survival of a small percentage of autografted glandular cells capable of regeneration as described above may be of great significance At the present time the relation of viability to successful homografting is not determined fully and may vary according to the type of tissue (185) The living elements of the homograft apparently fail to survive or regenerate in the host with rare exception. The grafted tissue is destroyed or devitalized gradually and may be replaced partially by host tissue To what extent method of preservation affects the physical constitution of a graft such as blood vessel or bone is not known The rate and extent of reaction however appear to be affected by method of preservation and by the degree of viability at implantation (186–188) Pierce and his associates (1949) (179) attributed lack of success with frozen aortic homografts to the loss of viable cells In the author's laboratory as in others functional success has been obtained with grafts shown by tissue culture to be nonviable at implantation (189) and Coleman *et al* (1955) (190) Viability of arterial homografts has been stated to be actually undesirable by Tate (1954) (191)

Non viable grafts of bone and blood vessels evidently have been used satisfactorily (151 192) Skin and glandular transplants and cornea on the other hand, need to be alive to function adequately The outgrowth of cells in tissue culture is undeniable evidence of cell survival. In spite of Carrel's suggestion in 1912 to determine viability of preserved tissue by *in vitro* cultivation the method was not prominently used for that purpose until the last decade At that tests have been limited almost entirely to skin and blood vessels (in addition to the endocrine work mentioned above)

The possibility of storing human skin in a viable state is of great clinical importance Fortunately this tissue is one of the hardiest. The epithelial cells and fibroblasts of chick embryo skin have been shown by tissue culture to survive at 0°C for 32 days (170) Adult rat and guinea pig skin survived for several days at room temperature although survival was longer at 5°C (175) Human skin can survive for many days at icebox temperatures. However tissue culture studies have shown that there is a progressive loss of viability during even very short periods of storage unless the tissue is placed immediately in a nutrient medium at a temperature between 0° and 6°C following its removal (104) Although some cells may survive a week of storage in saline in the icebox, there is a rapid drop in viability evident after 24 hours. Matthews (1945) (193) observed only a slight growth of fibroblasts from skin grafts preserved for eight days in saline-moistened gauze Growth of epithelial cells of the skin was gradually reduced during a week of storage at 4°C in a physiologic solution (103) This is not surprising since skin is a highly metabolic rapidly growing tissue

The reports of Hanks and Wallace (1949) (194) Marrangoni (1950) (195) and Allgöwer and Blocker (1952) (196) show a very poor percentage of growth from tissues stored in the absence of a nutritive medium. Hanks found that rabbit skin stored in shallow layers of 10 per cent serum at 6° to 8 C did not show a decline in viability over a 2 week period while skin stored in mineral oil showed a marked decrease Marrangoni using Hanks method, compared the viability of rabbit skin stored in 10 per cent serum, in pliofilm, and in the frozen state and correlated the tissue culture results with grafting results He reported that viability was best maintained with storage in serum He stated that he obtained excellent results with skin stored in serum for 4 to 6 weeks while pliofilm-wrapped and frozen grafts broke down. Allgöwer found that the survival

time of both rabbit and human skin as shown by tissue culture results was much longer with storage in serum than with storage in wet or Vaseline gauze. Grafts of both rabbit and human skin stored in serum were the most successful confirming the value of the tissue culture results. All demonstrable viability of the epidermis was lost after 12 days of storage in wet or Vaseline gauze, while the epidermis of skin stored up to 28 days in 10 per cent serum contained viable cells. There was a gradual decline in the amount of growth after a week of storage. Fibroblasts were more resistant to storage in gauze than was epithelium, the former showing slight growth after 2 weeks, when there was no growth of the epidermal cells. Allgöwer found that the conditions of serum storage were important.

In the author's laboratory growth has been obtained in tissue culture from human skin stored in a nutrient medium for over 6 weeks at 4 C In a comparison of the growth *in vitro* from skin preserved in 10 per cent serum or serum ultrafiltrate

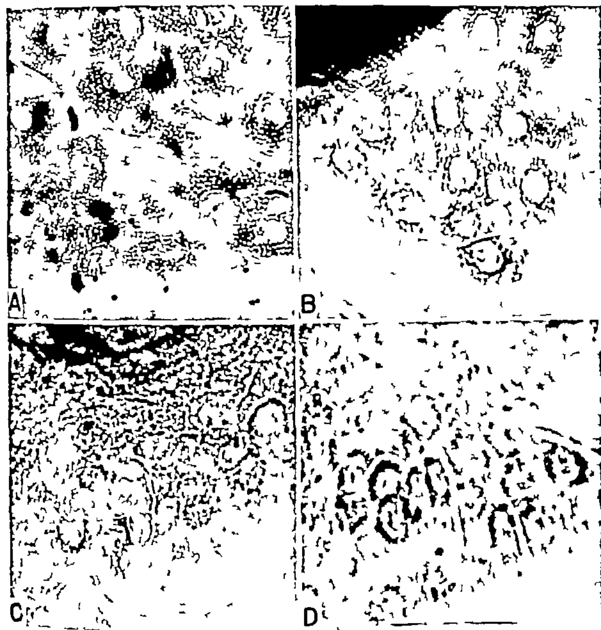



FIG 228. Effect of storage in 10 per cent human serum at 4 C on adult human Negro skin ×495 A. Living 4-day culture of fresh skin Note uneven distribution of pigment and mitotic figure upper right B Living 3-day culture of same skin stored for 2 weeks in autologous serum Growth rate and pigment distribution similar to control C Living 3-day culture of sister colony showing loss of pigmentation. D Living 8-day culture of same skin stored for 6 weeks Marked increase in lag period and irregular pigment distribution.

human arteries taken many hours postmortem and stored for several days in saline at icebox temperatures fail to grow in tissue cultures (104).

For many years tissues have been stored for use as grafts in moist air, Ringer's, Tyrode's or other physiologic solution, or in saline sponges or Vaseline gauze at icebox temperatures for short periods up to five or six weeks. Comparative grafting experiments correlated with tissue culture tests have indicated that viability can be maintained for much longer periods by storing the tissue in a nutrient medium, at least at the temperatures tested. This is not surprising, since such a medium provides nutritive materials and oxygen, buffers the acids, and dilutes toxic material.

The importance of viability as a factor determining the success or failure of grafts of large pieces of organized tissue has been much discussed. Freshly grafted tissue is not maintained in the host in an unchanged state. The percentage of the living elements of the autograft which survive is not known, but several studies indicate that viability of fresh autografts is reduced greatly following implantation (183, 184). The survival of a small percentage of autografted glandular cells capable of regeneration, as described above, may be of great significance. At the present time the relation of viability to successful homografting is not determined fully and may vary according to the type of tissue (185). The living elements of the homograft apparently fail to survive or regenerate in the host with rare exception. The grafted tissue is destroyed or devitalized gradually and may be replaced partially by host tissue. To what extent method of preservation affects the physical constitution of a graft such as blood vessel or bone is not known. The rate and extent of reaction, however, appear to be affected by method of preservation and by the degree of viability at implantation (186–188). Pierce and his associates (1949) (179) attributed lack of success with frozen aortic homografts to the loss of viable cells. In the author's laboratory, and in others, functional success has been obtained with grafts shown by tissue culture to be non-viable at implantation (189) and Coleman et al. (1950) (190). Viability of arterial homografts has been stated to be actually undesirable by Pate (1951) (191).

Non-viable grafts of bone and blood vessels evidently have been used satisfactorily (181, 192). Skin and glandular transplants and cornea, on the other hand, need to be alive to function adequately. The outgrowth of cells in tissue culture is undeniable evidence of cell survival. In spite of Carrel's suggestion in 1912 to determine viability of preserved tissue by in vitro cultivation the method was not prominently used for that purpose until the last decade. At that, tests have been limited almost entirely to skin and blood vessels (in addition to the endocrine work mentioned above).

The possibility of storing human skin in a viable state is of great clinical importance. Fortunately, this tissue is one of the hardiest. The epithelial cells and fibroblasts of chick embryo skin have been shown by tissue culture to survive at 0°C. for 32 days (176). Adult rat and guinea pig skin survived for several days at room temperature, although survival was longer at 2°C. (175). Human skin can survive for many days at icebox temperatures. However, tissue culture studies have shown that there is a progressive loss of viability during even very short periods of storage unless the tissue is placed immediately in a nutrient medium at a temperature between 0° and 6°C. following its removal (104). Although some cells may survive a week of storage in saline in the icebox, there is a rapid drop in viability evident after 24 hours. Matthews (1945) (193) observed only a slight growth of fibroblasts from skin grafts preserved for eight days in saline-moistened gauze. Growth of epithelial cells of the skin was gradually reduced during a week of storage at 4°C. in a physiologic solution (103). This is not surprising, since skin is a highly metabolic, rapidly growing tissue.

The reports of Hanks and Wallace (1949) (194), Marrangoni (1950) (195), and Allgöwer and Blocker (1952) (196) show a very poor percentage of growth from tissues stored in the absence of a nutritive medium. Hanks found that rabbit skin stored in shallow layers of 10 per cent serum at 0° to 8°C. did not show a decline in viability over a 2 week period, while skin stored in mineral oil showed a marked decrease. Marrangoni, using Hanks' method, compared the viability of rabbit skin stored in 10 per cent serum, in pliofilm, and in the frozen state, and correlated the tissue culture results with grafting results. He reported that viability was best maintained with storage in serum. He stated that he obtained excellent results with skin stored in serum for 4 to 6 weeks, while pliofilm-wrapped and frozen grafts broke down. Allgöwer found that the survival

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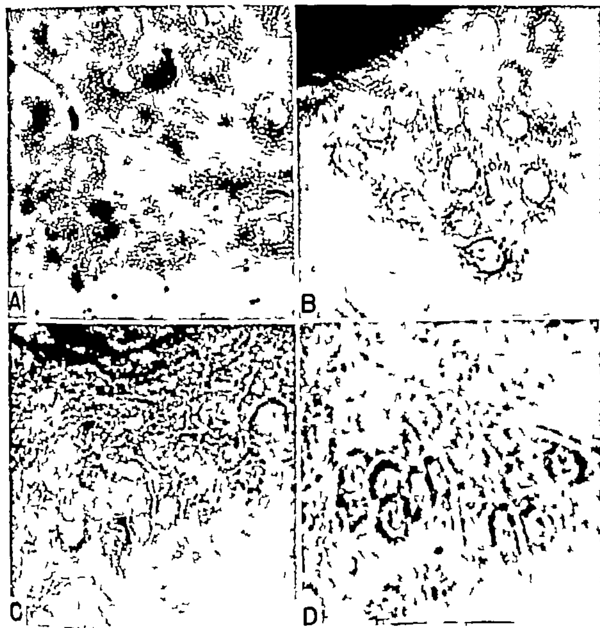



FIG. 238. Effect of storage in 10 per cent human serum at 4°C on adult human Negro skin ×495 A, Living 4-day culture of fresh skin Note uneven distribution of pigment and mitotic figure upper right B Living 3-day culture of same skin stored for 2 weeks in autologous serum. Growth rate and pigment distribution similar to control C Living 3-day culture of sister colony showing loss of pigmentation. D Living 8-day culture of same skin stored for 6 weeks Marked increase in lag period and irregular pigment distribution

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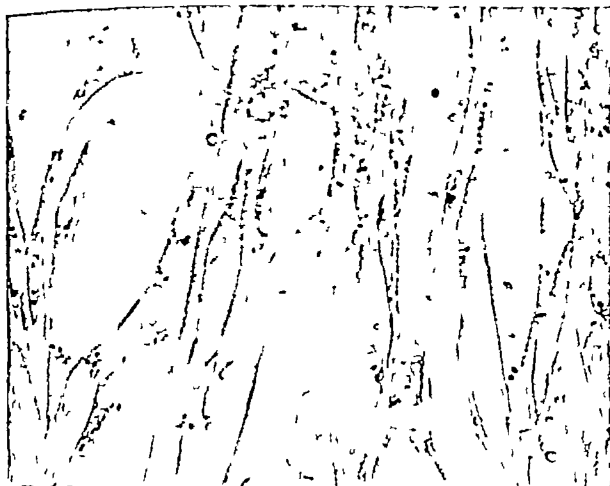




FIG 240 Living 13-day culture of abdominal aorta of adult dog stored for 26 days at 4 C in ox serum ultrafiltrate ×500

zero (178). Metabolism is slowed and oxygen demand is diminished at this temperature which on the other hand, is not low enough to kill the tissue. Replacement tissues have been stored mainly between 0° and 6°C but also in some instances in commercial deep freezers at about −27°C, and in carbon dioxide iceboxes at about −70°C. More recently when viability was not considered important they have been preserved in a lyophilized state. Masses of tissue do not survive at −27°C or when lyophilized, cell survival is negligible in large pieces of tissue stored at −70°C in the absence of a protective medium (179, 200–203) Although cell survival is significantly better at −70°C following partial dehydration, as observed originally by Luyet and his coworkers, it has not as yet been found to equal that of fresh tissue or that of the same tissue stored in a nutrient medium at a temperature just above zero (178 202, 204–208) There is also evidence from tissue culture that solutions containing concentrations of 10 per cent, 15 per cent, or more of protective media such as glycerol, the glycols, isopentane and glucose are in varying degrees, toxic to the tissues [unpublished experiments by Parshley and Simms also (27) (177) (202) and (209)]

Very marked is the individual variation in survival of the tissues of different animals under adverse conditions (178 202 210 211) Different types of cells withstand freezing temperatures to varying extents (176 197 211–213) The ability of tissue to withstand freezing temperatures varies also according to species (204–206 214) The importance of size as a limiting factor is discussed by Luyet and Gehenio (1940) (215) It is apparent from an examination of the literature that the tissues which have proved to be hardiest have been single cells or thin slivers of tissue for example some protozoa, spermatozoa tumor cells in suspension thin sections of epidermis, and isolated muscle fibers It seems unlikely therefore, that under the present conditions of storage at freezing temperature any great percentage of the cells of large masses of tissue will survive

Reports of the survival of replacement tissues

for one to 6 weeks with that from the same skin stored at −72°C in blood for the same time the former showed to advantage. The author's group obtained no growth from the frozen skin, while the skin stored in a nutrient medium maintained the same rate of growth as that of the control for 2 weeks. After this time viability declined but was still demonstrable after more than 6 weeks. Although there was no outgrowth of cells, there were indications of epithelial activity in the explant after 58 days of storage. After 2 weeks a deterioration of the tissue was indicated by a reduction in the number of colonies to grow from any piece of tissue and there was an increase in the lag period up to one to 2 weeks or more. Signs of degeneration such as loss of pigment in the cells of the outgrowth and the formation of pearl-like structures were observed in the cultures (fig 238). This observation is of interest in connection with reports by Taylor (1949) (197) of changes in the

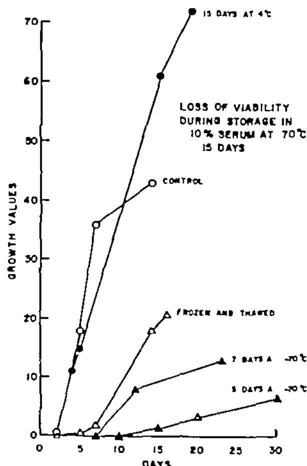



Fig 239 Increasing loss of viability of the racic ... of adult dog following freezing in 10 per cent serum at −72°C and storage at −70°C up to 15 days. Growth values are included for the same aorta stored at 4°C

pigmentation of rat skin following freezing and by Conway (1936) (198) who described loss of pigment in otherwise successful skin grafts. In the author's experiments autologous and homologous serum were found equally satisfactory. Placental serum was also used successfully. Serum ultrafiltrate was equally good up to 2 weeks and maintained viability up to 6 weeks although the growth was not as great as that *from the same* skin stored in serum. Earle and Evans (1950) (199) in collaboration with the Tissue Bank of the U. S. Naval Medical Center have reported consistent viability of adult human skin preserved in 10 per cent human serum in balanced salt solution up to 6 weeks and fluctuating result up to 8 weeks. The Tissue Bank considers this method superior to the saline gauze technique and reports successful results with autografts stored in this way for 84 days (181) (Hyatt *et al* 1952)

Adult aorta a relatively inert tissue with a low metabolic rate and less hardy than skin, can be maintained viable to some extent as shown by tissue culture refrigerated in a nutrient medium for a period of weeks (178–180). The significance for function of this limited number of cells mainly fibroblasts, has been questioned. In fact this low viability may be responsible for the relatively slow reaction of the host to the aortic homograft. However grafts stored at just above zero in the absence of a nutrient become rapidly unsuitable for use (179) and viability may well be an indicator of the physical state of the graft. Pierce was able to demonstrate fibroblastic growth from vessels stored up to seven weeks. The author's group found 81 per cent of dog aortas stored in 10 per cent serum at 4°C up to one month produced *outgrowths of fibroblasts* and some endothelial cells and 21 per cent of those kept up to 6 weeks contained living cells (178). Vessels maintained for a week grew as well as or better than the controls (fig 239). After longer storage the amount of growth decreased markedly and the lag period increased. Homologous serum was as satisfactory as autologous serum as a preservative. Serum ultrafiltrate has maintained viability equally well for periods of several weeks and may be preferable to serum as a short term storage medium since it is free of specific protein and lipid (fig 240).

A large number of tissue culture experiments concerned with the effect on viability of extreme ranges of temperature have indicated that the optimal storage temperature is slightly above

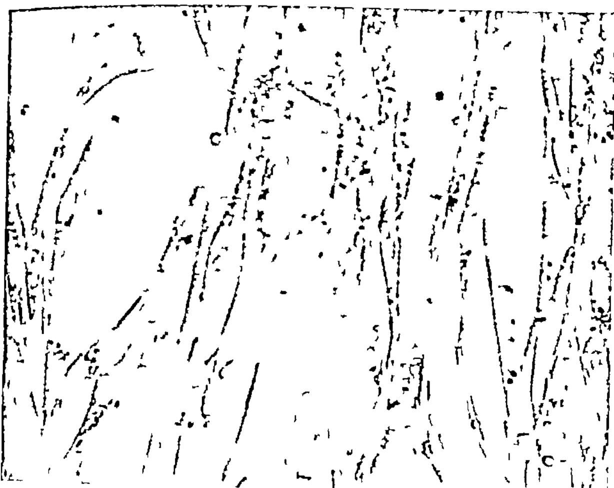



FIG 240 Living 13-day culture of abdominal aorta of adult dog stored for 26 days at 4°C in ox serum ultrafiltrate ×500

zero (178) Metabolism is slowed and oxygen demand is diminished at this temperature which on the other hand is not low enough to kill the tissue. Replacement tissues have been stored mainly between 0° and 6°C but also in some instances in commercial deep freezers at about −27°C, and in carbon dioxide iceboxes at about −70°C. More recently when viability was not considered important they have been preserved in a lyophilized state Masses of tissue do not survive at −27°C or when lyophilized cell survival is negligible in large pieces of tissue stored at −70°C. in the absence of a protective medium (179, 200–203) Although cell survival is significantly better at −70°C following partial dehydration, as observed originally by Luyet and his coworkers, it has not as yet been found to equal that of fresh tissue or that of the same tissue stored in a nutrient medium at a temperature just above zero (178 202 204–208) There is also evidence from tissue culture that solutions containing concentrations of 10 per cent 15 per cent or more of protective media such as glycerol, the glycols isopentane and glucose are in varying degrees toxic to the tissues [unpublished experiments by Parshley and Simms also (27) (177) (202) and (209)]

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Reports of the survival of replacement tissues

for months or possibly indefinitely at subzero temperatures are based almost entirely on transplantation results. The success of these grafts has not been proved to depend primarily on the presence of viable cells. There is no doubt that a number of embryonic and adult tissues and particularly tumor cells may survive extremes of temperature from −70° to −250°. Tumor cells have retained their ability to induce tumors during storage at subzero temperatures for one to two years. However, the percentage of surviving cells has been estimated to be as small as 1 per cent (216). Experiments with normal adult tissue have not extended over many days as a rule. However, some workers have noted excellent results with tissue stored for several months following pretreatment with a protective medium. Billingham and Medawar (1952) (217) reported the successful autotransplantation of rabbit skin which had been stored for 4 months at −70°C following freezing in isopentane at −150°C. In some instances the skin was soaked for an hour in 15 per cent glycerol before freezing. Autografts of dog skin stored at −70°C for several months following partial dehydration with ethylene glycol and freezing in liquid nitrogen survived according to Kelly and his associates (1952) (203). It should be noted, however, that in these studies no direct tests for cell viability were made. In both instances the tissues frozen were extremely

TABLE 25

*Loss of viability of adult dog thoracic aorta following rapid freezing and storage at −70°C*

| Time at −70°C | In 10% Dextrose | | | In 10% Serum | | | Percentage Viable Segments |
|---|---|---|---|---|---|---|---|
| | Total | No. Viable | Av. Lag (before growth) | Total | No. Viable | Av. Lag (before growth) | |
| | | | *days* | | | *days* | *per cent* |
| Controls | 5 | 5 | 3 | 4 | 4 | 3 | 100 |
| 30 sec | 5 | 4 | 7 | 4 | 3 | 5 | 78 |
| 3 hr | 1 | 1 | 10 | | | | |
| 1 day | 3 | 2 | 12.5 | 3 | 1 | 12 | 62 |
| 2 days | 2 | 1 | 9 | 2 | 1 | 10 | |
| 3 days | 1 | 1 | 10 | 1 | 1 | 10 | |
| 5 days | 1 | 0 | | 1 | 0 | | |
| 6 days | | | | 1 | 0 | | 40 |
| 7 days | 1 | 1 | 13 | 1 | 1 | 10 | |
| 8 days | 1 | 0 | | 1 | 0 | | 25 |
| 15 days | 1 | 1 | 15 | 1 | 0 | | |




FIG. 211. A living 21-day culture of adult dog aorta frozen in 10 per cent dextrose at −70°C and thawed at once. The cells are thin, binucleate cells (B) are frequent but otherwise cells are normal in appearance. ×250

than Chamness (1956) (218) observed a gradual decrease in enzyme activity of skin stored in the icebox in saline gauze, with complete loss at 8 weeks. Freezing and thawing regardless of method or speed, caused considerable loss Pretreatment with 15 per cent glycerol lactate gave partial but inconstant protection. Earle and his coworkers (1956) (199) were unable to obtain growth *in vitro* from human skin pretreated with 15 per cent glycerine, quick frozen, and stored at dry ice temperature, although this tissue served well as a clinical dressing after storage up to 18 weeks. Rabbit cornea frozen for an hour and thawed following similar treatment with 15 per cent glycerol consistently produced a good growth in cultures of epithelium and fibroblasts after a slightly increased lag period (219)

Swan and his coworkers (1952) (202) obtained growth in tissue culture from about 50 per cent of sections of dog aorta, tissue several millimeters thick after freezing at −79°C in 10 per cent serum and thawing Only 4 out of 12 vessels stored at this temperature up to 3 weeks contained viable cells however Tissue culture experiments in the author's laboratory (178, 220) showed that, although almost 80 per cent of segments of dog aorta frozen at −70°C in either 10 per cent dextrose or 10 per cent serum were viable when thawed immediately only 40 per cent were found to contain living cells after a week of storage at −79°C and only 25 per cent, after 15 days, indicating a progressive deterioration at this temperature (table 25) These experiments show that a certain percentage of the cells




FIG 242. A living 21-day culture of the same aorta as in figure 241 frozen in the same way and stored for 15 days at −70°C The outgrowth is sparse and the cells appear shrivelled Note odd shapes binucleate cells (B) vacuoles (V) fragmenting cell membrane (F) and debris ×250

of adult dog aorta survive freezing at −70°C in a medium of either 10 per cent serum or 10 per cent dextrose but the number of viable cells is less than that of the fresh tissue Although a little over three-quarters of the aortic rings survived the freezing treatment described, the number of viable explants was reduced greatly as compared with those of the fresh tissue Storage at −70°C resulted in a gradual loss of viability which was strikingly greater than that of aorta stored for the same period of time in 10 per cent serum at 4 C (fig. 239) Storage for one to 2 weeks in 10 per cent serum at 4 C did not have any marked effect on the rate and character of the outgrowth in tissue culture Injury from freezing even in a protective medium was immediate as indicated by the effect on the rate of growth *in vitro*

Dog aorta a fairly inert tissue when fresh does not appear to survive freezing as well as does skin. Species and tissue differences may be responsible as well as the thickness of the tissue The primary effects of storage as observed in tissue cultures which indicate cell death or injury or physiologic change are a tremendous increase in the latent period (before the onset of growth) of as much as 2 or 3 weeks and a reduction in the number of explants which grow The tissue may react differently from the fresh tissue to culture media. With increased injury the cell outgrowth may be initially sparse The cells appear shrivelled and

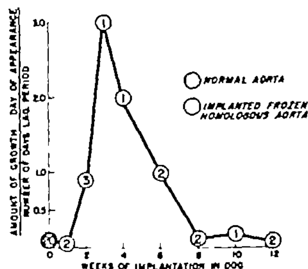



FIG 43 Effect of length of time of implantation on growth rate in tissue culture of homograft of an adult dog aorta following removal from the host

degenerate readily Difference in size and shape may be marked. Cells with vacuoles and blebs are observed and there is a great deal of debris (figs 241 and 242) At the same time mitotic figures may be numerous and the outgrowths eventually may become luxuriant. The few surviving fragments of a piece of relatively non viable tissue may grow even better than the controls The relatively low percentage of cell survival and the evidence of progressive change signifying tissue injury during long storage observed by the author and others indicate that in spite of great progress toward the development of a method of preservation at low temperatures of tissues for grafting, at the present time no procedure is adequate for the permanent storage of large sections of tissue in an unchanged condition. While the survival of a few cells may be sufficient possibly for the success of certain types of graft it is doubtful whether it is enough to insure the function of large homografts of highly organized tissues.

## REMAINING PROBLEMS

Problems still unsettled concerning the fate of grafted tissue the answers to which will be to some extent particular to a given type of graft, may be listed as follows 1) To what extent does the graft tissue survive? 2) To what extent and at what rate is it replaced by host tissue? 3) Is there a mutual reaction between graft and host? 4) Is this reaction solely one of actively acquired immunity humoral or cellular or both? 5) Is the reaction limited by individual or species differences? Direct microscopic observation of grafts implanted in the living animal by means of transparent chambers has contributed a great deal of pertinent information. Williams (10 3) (183) has given a detailed account of conditions of survival over long periods of time of tiny fragments of 20 different tissues autografted and homografted in rabbit ears Of the autograft five failed to survive adrenal medulla ovarian follicles brain cortex, pancreas and red bone marrow Four tissues survived partially adrenal zona fasciculata sympathetic ganglion epidermis and spermatogenic tubules Eleven survived. These were omentum, thyroid adrenal zona glomerulosa interstitial cell of the ovary bone parathyroid, brown fat abdominal fat spleen red pulp lymph node and testicular interstitial cell which were observed to develop from connective tissue and to revert to it Eleven of 15 homografted tissues failed to survive The re were great



Figs. 244–247 Living cultures of homograft of adult dog aorta stored in air at −70°C for two weeks. …e graft was non viable at implantation and was removed after 10 days in the host. Adjacent host …rta is also shown. ×245

Fig. 244 (above left). Four-day culture of distal end of graft showing leukocytic infiltration, a few …roblasts and debris.

Fig. 245 (below left). Four-day culture of adjacent host tissue showing massive leukocytic infiltra…on, degenerate cells and debris.

Fig. 246 (above right). Another 4-day culture of distal end of graft showing invasion of endothelial …lls.

Fig. 247 (below right). Same culture 10-days old. Sheet of endothelium.

variation in persistence of different tissues, survival of the same tissue and degree of leukocyte invasion in different animals. An epidermoid carcinoma and grafts of choroid plexus and ciliary process survived. There was no difference other than in time between autografts and homografts. There was no correlation between graft survival and white cell infiltration. Cortisone had no effect in prolonging the life of homografts of thyroid adrenal fasciculata or spleen. The grafts assumed the structure and organization characteristic of the source tissue. Failure to survive was considered due in the first instance to inability of the transplanted cells to function adequately rather than to a defense mechanism of host or region.

Recent modification of the transparent chamber technique by Algire and his coworkers (221 222) has made possible separation of grafted tissue and cells from host cells and blood supply by means of membrane filters. Homografts will survive for months in immunized mice in these chambers which permit diffusion of essential metabolites but not of cells and which are in effect a tissue culture *in vivo*. Mouse tissue "target" cells or combinations of these cells with washed splenic cells from target immunized mice inside similar diffusion chambers were placed intraperitoneally in isologous or homologous mice or rats some of which had been immunized previously to the target cells. Some chambers excluded host cells and others permitted entry of host leukocytes and macrophages. Results indicated that homografts in mice are usually destroyed by close association with "immunized" host cells (prob-

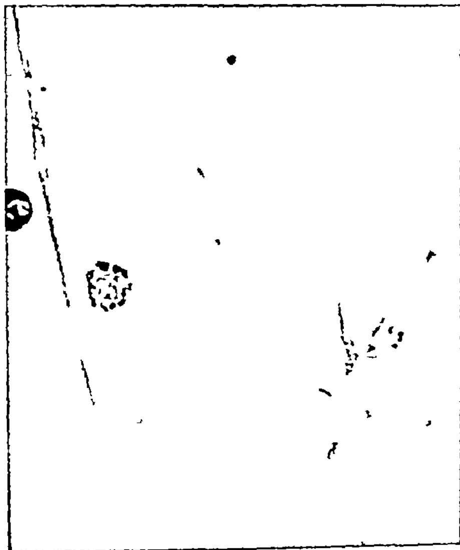


FIG 218 Living 25-day culture of homograft of adult dog aorta viable at implantation after 1½ years in host. Sparse macrophages and fibroblasts containing orange fat globules and pigment reflect atherosclerotic condition of graft ×490

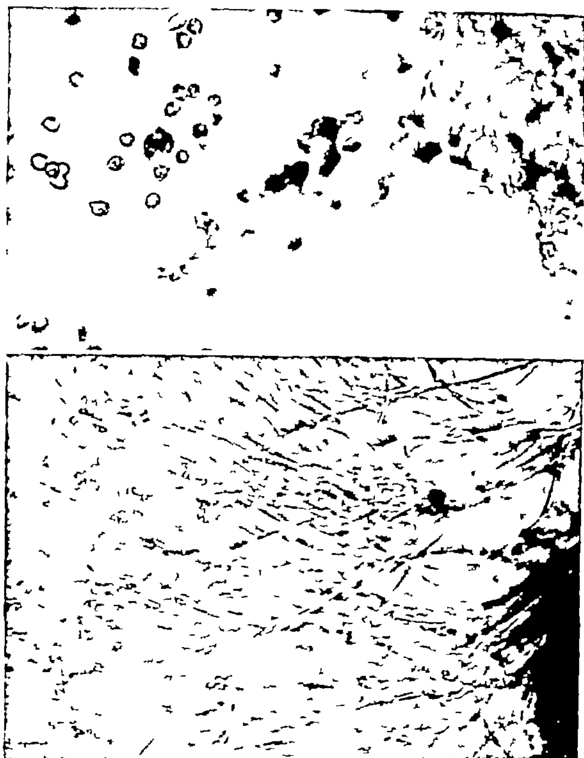
ably by contact with lymphocytes) This conclusion is considered compatible with the hypothesis that antibodies to homografts are transported by lymphocytes. Attempts to demonstrate cytotoxic action in tissue culture of serum or host tissue of homografted animals or immunized lymph node and spleen (rabbits) have failed (223 224) However Allgower found that heterologous skin extract (human, injected into rabbits) caused formation of strong cytotoxins which killed human epidermal cells *in vitro* It is possible that the negative tissue culture results with homografts were due to too low a concentration of the cytotoxic factor or the absence of cofactors present under systemic conditions. However this technique offers certain advantages for the analysis of the mechanism of graft rejection and for attempts to modify the reaction.

Tissue culture has received little notice as a means of study of the graft following implantation. The condition of the graft is reflected by the rate and type of growth which occurs *in vitro* on removal. In the author's experiments with homologous grafts of dog aorta the same kind of growth *in vitro* was obtained from vessels shown to be both viable and non viable (frozen) at the time of implantation. There appeared to be no relation between degree of viability at implantation and amount of growth following removal. Grafts which grew poorly gave excellent functional results and *vice versa*. The growth from grafts removed from the host after one day to two weeks was scant never more than half that of the tissue at implantation and considerably less than that of the adjacent host (fig. 243) It was predominantly of leukocytes, including dwindled polymorphonuclears, monocytes, and macrophages, but also included a few fibroblasts and endothelial cells. There was a marked increase in the lag period before onset of growth Vessels removed between the second and sixth week grew rapidly with a shorter lag and greater rate of growth than average fresh aorta. The growth consisted of fibroblasts and endothelial cells and variable numbers of leukocytes, at times in pure culture (figs. 244 to 247) Small degenerating leukocytes were numerous. Dead cells, debris, and occasionally fat globules were evident. The adjacent host tissue showed the same type of growth indicating a mutual reaction to some extent.

In all instances in which proximal middle, and distal thirds of the grafts were cultured separately the earliest and greatest growth occurred from the ends. This was characteristic of all grafts regardless of length of implant. After 6 weeks the rate of growth declined to that comparable to fresh aorta. The persistence of leukocytes in the cultures varied considerably They were occasionally present in cultures of grafts implanted up to a year After 3 months of implantation the tissue grew very slowly and usually scantily Small leukocytes were not observed but macrophages were. A series of homografts which had shown excellent function for 18 months to 4½ years following implantation was cultured. There was a tremendous reduction in growth compared to that of the fresh tissue. None of these grafts showed growth equal to 50 per cent of that from the tissue when originally obtained. The growth from the adjacent host aorta, though significantly greater was about half that of fresh aorta. Leukocytes were found to be numerous in cultures of only three of the grafts, which were atherosclerotic. Great numbers of large granular macrophages were present in these, containing pigment and yellow fat globules (fig 248) Endothelium grew out in capillary sprouts from a graft removed from the host after 3½ years (fig 249) Three grafts stored in glass ampules following electronic irradiation in contrast to the nutrient and frozen grafts, gave evidence of marked reaction when cultured after implantation for 6 9 and 18 months. They produced almost pure cultures of granular leukocytes, engorged macrophages, and giant cells (figs. 250–251) Similarly autogenous



FIG 249 Living 9-day culture of homograft of adult dog aorta viable at implantation and removed after 3 years and 7 months in host Note outgrowth of endothelial sprouts from distal end of graft This graft was thin but in good condition ×368



FIGS 250–251 Living 25-day culture of aortic homograft electronically irradiated (1–6 million rep) and stored for 37 days at −70°C The graft was non viable at implantation and was removed after 9 months in the host dog Adjacent host tissue also shown Both grew very well ×200

FIG 250 (*above*) Graft tissue. Dense leukocytes and a few fibroblasts

FIG 251 (*below*) Host tissue Dense fibroblast scattered macrophages and debris



FIG 252 Living 6-day culture of autologous fascial tube removed after 10 months of implantation in dog aorta Dense outgrowth of fibroblasts and macrophages ×120

grafts of fascial tubes gave evidence of extensive leukocytic infiltration when removed nearly a year after implantation, and they produced cultures containing many macrophages loaded with debris, pigment and fat (fig 252)

These observations are of interest since they indicate little relation between viability and satisfactory function in the case of the homologous aortic graft. There is a tremendous cellular loss which increases with time and an attempt at replacement by host tissue. This replacement is limited to fibrous tissue and endothelial cells Leukocytic infiltration varies considerably reflects a mutual reaction between host and graft and appears to be intensified by certain types of tissue preservation. The reaction varies among individuals and is in several instances marked against grafted autologous tissue. An examination of the combined *in vivo* and *in vitro* observations would indicate that the reaction to grafted tissues is a highly complex one involving a variety of factors, not the least of which are individual and tissue differences.

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